SYNTHESIS, CHARACTERIZATION AND IN-VITRO ANTI-OXIDANT ACTIVITY OF SOME 1,4-DIHYDROPYRIDINES AND THEIR MANNICH BASES

A Dissertation submitted to

THE TAMIL NADU Dr.M.G.R.MEDICAL UNIVERSITY,
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In partial fulfillment for the award of degree of

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Submitted by
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Dedicated To
My Beloved Parents
and Teachers

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I bow to my affectionate my parents and brothers & sister for their blessings, support, love and prayers.

ARUMUGA NAVAMANI. K.

LIST OF ABBREVIATIONS

mg	Milligram
%	Percentage
wt	Weight
hr	Hour
^{o}c	Degree Centigrade
>	Greater
<	Lesser
std	Standard
DMSO	Dimethyl sulphoxide
TLC	Thin layer chromatography
KBr	Potassium bromide
m.p.	Melting Point
R_f	Retention Factor
µg	Microgram
mL	Millilitre
µL	Microlitre
HO	Hydroxylion
^{1}HNMR	Proton Nuclear Magnetic Resonance
IR	Infrared Spectroscopy
TMS	Tetra methyl silane

MS	Mass Spectroscopy
TCA	Tricarboxylic acid
BHT	Butylated Hydroxy Toluene
EDTA	Ethylene diamine tetra acetic acid
q.s.	quantity sufficient

LIST OF FIGURES

LIST OF TABLES

CONTENTS

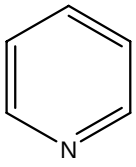
INTRODUCTION

INTRODUCTION

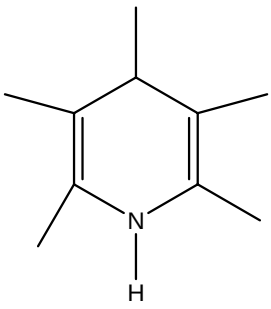
For small organic molecules, simple nitrogen containing heterocyclic receive a large amount of attention in the literature, as a consequence of their exciting biological properties and they role as pharmacophores of considerable historical importance.

Hetero cyclic compounds are stable cyclic compounds in which at least one atom other than carbon forms a part of the ring. The hetero atom is mostly nitrogen, oxygen or sulphur. The heterocyclic are usually five or six member cyclic compounds

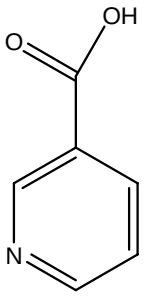
Dihydropyridine[1] is one of the heterocyclic compounds, which is simple derivative of pyridine. They are particularly well known in pharmacology as L-type calcium channel blockers. They are readily convertible to pyridines and are important role as intermediates in reactions of pyridine e.g in nucleophilic substitutions and reductions as well as acylations in the presence of pyridine. Dihydropyridines are of utmost importance in biological systems especially NADH2 which is involved in the biological redox reactions. It is water soluble B complex vitamin.



Pyridine



1,4-dihydropyridine

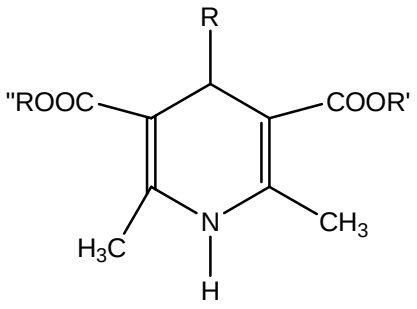


Nicotinic acid

1, 4-dihydropyridine

Physical properties

Structure



R-Hydroxy phenyl

R'-$COOC_2H_5$

R''-$COOC_2H_5$

Description

Dihydro pyridine is an amphoteric, colourless with a distinctive unpleasant fish-like odour. The pyridine ring occurs in many important compounds, including nicotinamides.Its PH in aqueous saturated solution is 8.5.

Solubility

It is freely soluble in boiling water, alcohol, alkali hydroxide, carbonates and in propylene glycol .Ii is practically insoluble in diethylether.

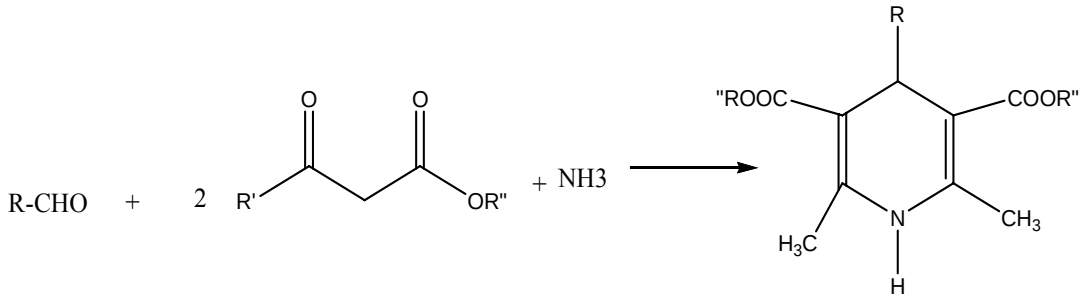
Sources

It occurs in all living cells in small amounts.

Chemistry

The molecular formula is **C_5H_7N.**

HANTZSCH DIHYDRO PYRIDINE (PYRIDINE) SYNTHESIS

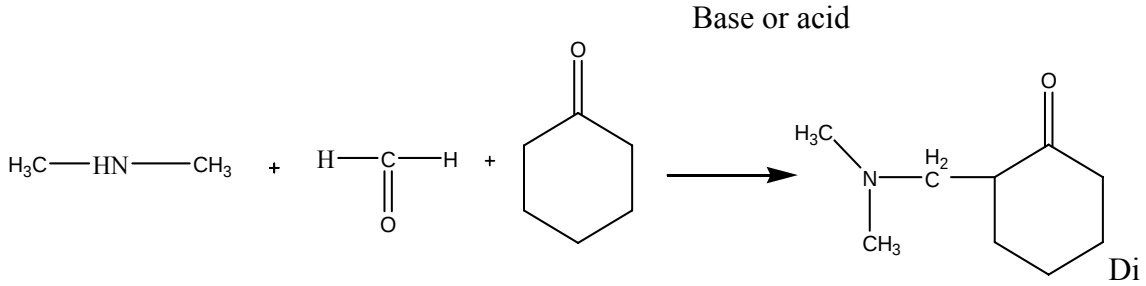


This reaction allows the preparation of dihydro pyridine derivatives by condensation of an aldehyde with two equivalents of a beta -ketoester in the presence of ammonia. Subsequent oxidation (or dehydrogenation) gives pyridine-3, 5-dicarboxylates, which may also be decarboxylated to yield the corresponding pyridines.

Mannich bases[2]

A Mannich base is a β-amino ketone, which is formed by reacting of an amine, formaldehyde (or an aldehyde) and a carbon acid. The mannich base is an end product in the mannich reaction, which is a nucleophilic addition reaction of a non-enolizable aldehyde and primary or secondary amines to produce resonance stabilized imines (minimum ion or imines salt). The addition of a compound containing active hydrogen atom to the schiff's base gives the Mannich base.

The essential feature of the Mannich reaction is the replacement of the active hydrogen atom by an amino methyl group.

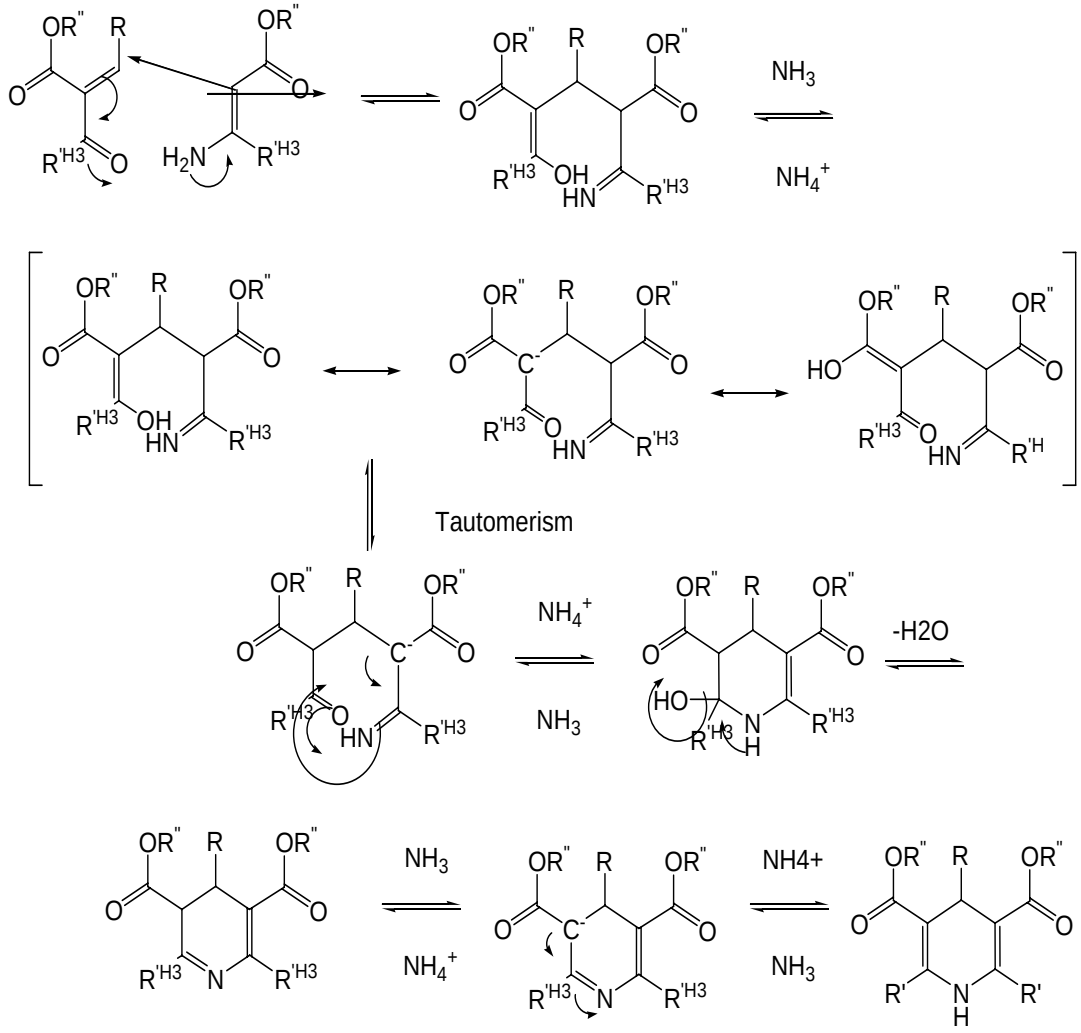


methylamine Formaldehyde Cyclohexanone Mannich base

Mannich bases are important compounds owing to their wide range of biological and industrial applications. They are also employed as intermediates in chemical synthesis and polymer chemistry. Several important therapeutic compounds have been synthesized via the Mannich reaction. They have also been found to possess pharmacological activities, such as asanticancer, antiviral, anti-malarial, anti-tubercular, anti-bacterial, analgesic, anti-inflammatory activity etc [3].

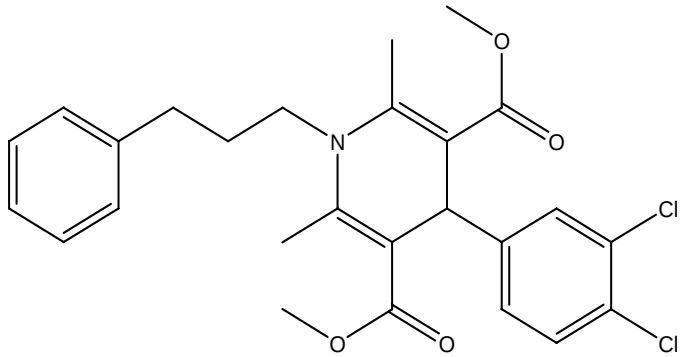
Mechanism of the Hantzsch Dihydropyridine synthesis[4]

The reaction can be visualized as proceeding through a knoevenagel condensation product as a key intermediate.



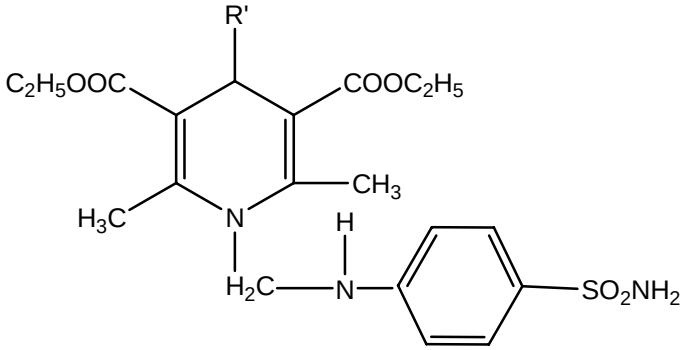
Derivatives of 1, 4-dihyropyridine[5,35]

M_1



Dimethyl1,4-dihydro-4-(3,4-dichlorophenyl)-2,6-dimethyl-1-(3-phenylpropyl)-3,5-pyridine dicarboxylate

M_2



3,5-diethoxycarbonyl-1-[(4'-sulfamoyl-1'-aminomethyl)phenyl)-1-4-dihydro-2,6-dimethyl-4-(3'-methoxy-4'-hydroxy phenyl)-pyridine.

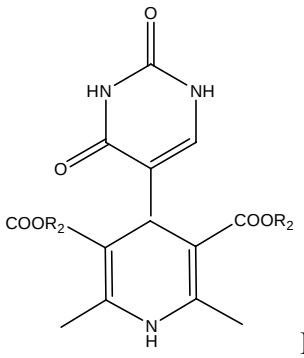
The dihydropyridine derivatives have variety of pharmacological activities like Analgesic, Anti-inflammatory, Anti-fungal, Anti-bacterial, Anti-convulsant, Anti-hypertensive.

Due to importance of 1, 4-dihydropyridine derivatives and its isomers, the aim of this dissertation is to evaluate the invitro antioxidant activity and antimicrobial activity against staphylococcus auereus of several compounds of this class.

REVIEW OF LITERATURE

Bu chanan *et al*[6]., synthesised the structures of a number of mannich bases which have been checked by NMR1 the deshielding effect of N-protonation being used to identify adjacent protons.whilist mannich bases lacking a beta- proton can react Michael- wise via a rearrangement, but their quaternary methiodides do not. On this evidence, anomalous literature reports can be rationalized.

Jungjin suh *et al*[7]., synthesised and carried out the anti hypertensive activity of 4-(2,4-Dioxo-5-pyrimidyl)-1,4-dihydro pyridine.



R-C_2H_5

Denner *et al*[8]., synthesised and carried out biological evaluation of new 1, 4-dihydro pyridines as anti-hypertensive agents in rats.

Kawashima.Y *et al*[9]., synthesised and performed anti-hypertensive activities of 1, 4-dihydropyridine-5-phosphonate derivatives.

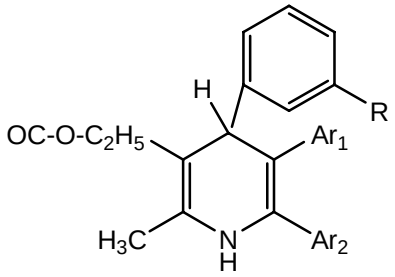
JAM Christians *et al*[10]., synthesised and carried out in vitro pharmacology of new 1,4-dihydropyridines.2-(w-aminoalkylthiomethyl)-1,4-dihydropyridines as potent calcium channel blockers.

J M Vierfond *et al*[11].,reported the synthesis, binding affinity and antioxidant activity of 1,4-dihydropyridine.

R- H

Ar_1 -2-pyrazinyl

Ar_2 -2-pyrazinyl

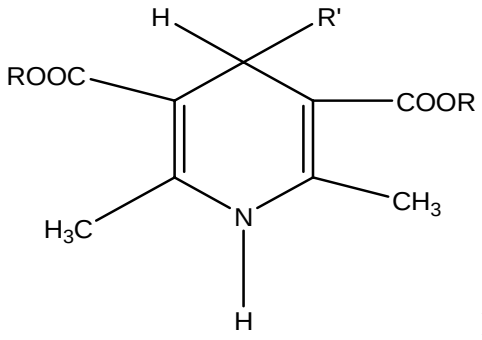


M.Amir *et al*[12]., reported 6-phenyl-1, 4-dihydro pyridine derivatives as potent and selective A3- adenosine receptor antagonist.

Kotecka *et al*[13].,we had compared the ex-vivo anti-malarial activity of 12 new quinoline di-mannich base compounds containing the 7-dichloroquinoline (or) 7-trifluoro methylquinoline nucleus with amodiaquine, chloroquine and pyronaridine using the saimiri- bioassay model.In vitro activity against the multi drug-resistantK1 isolate of plasmodium falciparum was determined in serum samples by measuring the maximum inhibitory dilution at which the treated monkey serum inhibited schizont maturation in vitro of the 12 mannich bases tested, 8 were associated with levels of ex-vivo anti-malarial activity in serum greater than those of amodiaquine, chloroquine, or pyronaridine 1to 7 days after drug administration. Further studies were carried out with four of these compounds, and results showed.

G.Diaz-Araya *et al*[14]., synthesised and carried out the antioxidant effects of 1,4-dihydro pyridine and Nitroso aryl derivatives on the Fe3+/ascorbate- stimulated lipid per oxidation in rat brain slice.

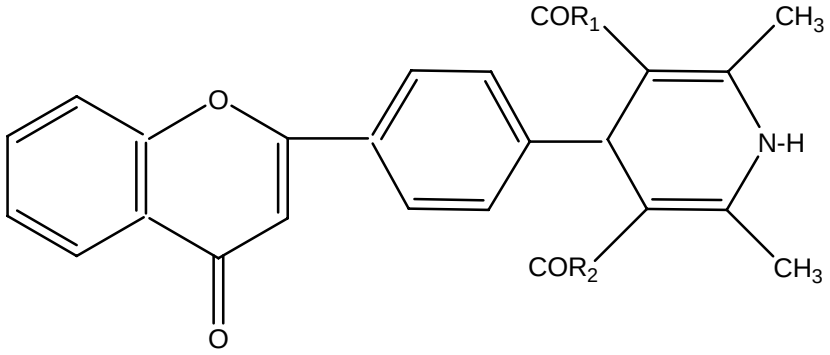
Tirzitis *et al*[15].,synthesised some 2, 6-dimethyl-3, 5-dialkoxy carbonyl-1,4-dihydro pyridines in metal-ion catalyzed lipid per oxidation and screened for antioxidant activity.



R-H

R'-OCH_3

Kruk *et al*[16].,performed the antioxidant activity of 4-flavonil-1, 4-dihydro pyridine derivatives.



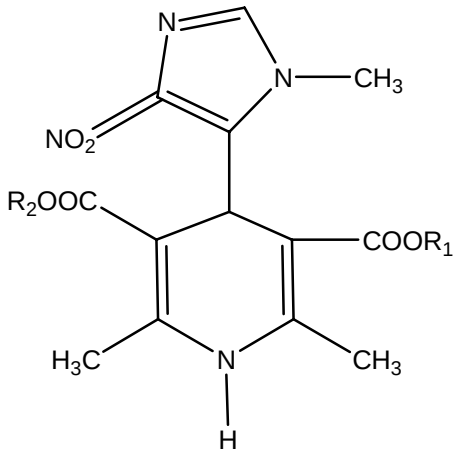
$R_1 - OCH_3$

$R_2 - OCH_3$

Pandeya *et al*[17].,synthesised and done the spectral characterization, in vitro antibacterial and antifungal activities of N-mannich bases of 3(N-pyrimethaminylimino) isatin.

Khan *et al*[18].,studied the mechanism- based inactivation of thioredoxin reductase from plasmodium falciparum by mannich bases implication for cytotoxicity.

Shafiee *et al*[19].,reported the anticonvulsant activities of new 1, 4-dihydropyridine derivatives containing 4-nitroimidazolyl substituents.



R_1-CH $_2C_6H_5$

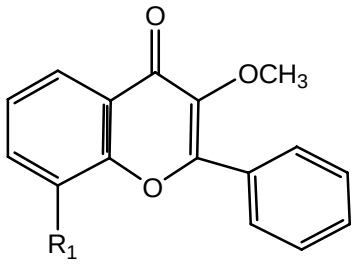
R_2- CH_3

Lopes *et al*[20].,synthesised some novel N-mannich base-type derivatives of the antimalarial drug amodiaquine and their reaction with tertiary N-chloromethylamides with the exception of the derivative of ethyl hippurate, all the so-formed (1-amidomethl-1H-quinolin-4-ylidine) arylamines displayed high chemical and enzymatic stability. These compounds displayed antimalarial activity against the multi-drug resistant plasmodium falciparum strain Dd2 (IC 50 values 15-31nm) and demonstrated no significant loss in activity compared to amodiaquine.

Dinh Thanh Hai *et al*[21].,synthesised (5-nitrofurfural and m-nitro benzaldehydes) by the condensation of aromatic aldehydes with hydantoin. The derivatives I and VI were obtained. The compounds I VI underwent mannich reaction and gave 8 mannich base derivatives (II-V, VII-X). The structures of synthesised compounds have been characterized by IR, UV, and ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. The obtained compounds were tested for biological activities such as antibacterial, antifungal and anticancer. Compounds I and its mannich base derivatives showed high biological activities.

R.Sridhar, P.T.Perumal *et al*[22].,reported a new protocol to synthesize 1,4-dihydropyridines by using 3, 4, 5-trifluorobenzenboronic acid as a catalyst in ionic liquid and synthesis of novel 4-(3-carboxyl-1H-pyrazol-4-yl)-1,4-dihydropyridines.

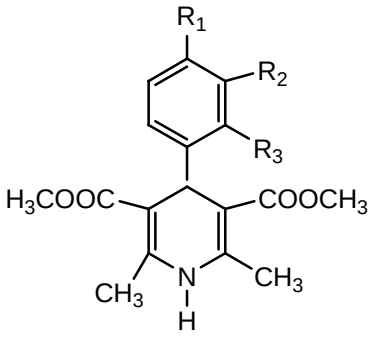
R.Budriesi *et al*[23].,synthesised the 1,4-dihydropyridine derivatives as calcium channel modulators: the role of 3-methoxy- flavones moiety was found to be angina and antihypertensive activity.



R1-$CH_2CH{=}CH_2$

Negm Nobel's *et al*[24].,prepared some novel series of cationic surfactants based on mannich base (produced from the condensation of piperidine and / or morpholine as secondary amine and para formaldehyde in the presence of 8- hydroxyl quinoline).The chemical structures of the synthesised cationic surfactants were confirmed using elemental analysis, FTIR spectroscopy and ^{1}H NMR. Surface activities of the prepared surfactants were measured including surface tension (gamma), critical micelle concentration effectiveness (Pi(cmc)), efficiency(Pc 20), maximum surface excess (Gamma(max), minimum surface area(A(min), interfacial tension(gammaIT)), emulsification power and foaming power at 25˚c. The structural influences on their surface activities and adsorption free energy were discussed.

A.K.Chhillar *et al*[25].,reported microwave-assisted synthesis of antimicrobial dihydropyridines and tetrahydropyrimidin-2-ones and some novel compounds against *aspergillus's niger*.



R_1- OCH3

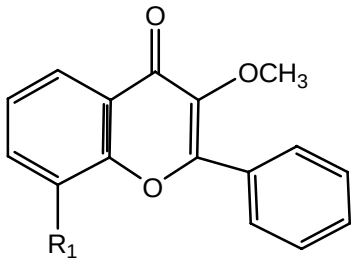
R_2-H

R_3-H

F.Mustata L.Bicu *et al*[26].,synthesised and characterization of p-amino benzoic acid/Cyclohexanone/formaldehyde resins as hardner for epoxy resins.

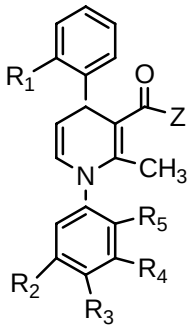
M.A. Zolfigol *et al*[27].,synthesised iodine catalyzed synthesis of novel hantzsch N-hydroxyethyl 1,4-dihydropyridines under mild conditions.

SR Pattan *et al*[28].,the synthesised and evaluation of some new substituted 1,4-dihydropyridine derivatives and their anti-inflammatory activity.



R1-$CH_2CH{=}CH_2$

V.Sridharan *et al*[29].,synthesised some new 3-component domino of 1,4-dihydropyridines.



R_1-H

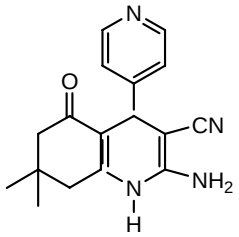
R_2-OCH_3

R_3-CH_3

R_4-CH_3

R_5-CH_3

R. Leon *et al*[30].,synthesised 6-amino-1,4-dihydropyridines that prevent calcium overload and neuronal death to posses antihypertensive activity.



J Carbajo *et al*[31].,synthesised and carried antihypertensive activity of some C-substituted 2, 6-dimethyl-1,4-dihydropyridines.

M. Ashok *et al*[32].,performed convenient one pot synthesis and antimicrobial evaluation of some new mannich bases carrying 4-methylthio benzyl moiety.

Belle Ds and Singhvi *et al*[33].,performed synthesis and antimicrobial activity of some mannich bases of 6-substituted-2-amino benzothiazole.

Rakeshkumar *et al*[34]., synthesised and performed antimicrobial activity of 4-(5-chloro -3-methyl-1-phenyl-1H-pyrazol-4-yl)–dihydropyridines and 4-(5-chloro -3-methyl-1-phenyl- 1H-pyrazol-4-yl) – 3,4-dihydropyrimidin-2-ones.

BBSubudhi *et al*[35].,performed the synthesis and antiulcer activity study of 1,4-dihydropyridines and their mannich bases with sulphanilamide.

OBJECTIVE AND PLAN OF WORK

The Dihydropyridine have already proven to have variety of pharmacological activities like analgesic, anti-inflammatory, anti-fungal, anti-bacterial, anti-oxidant, anti-convulsant, anti-hypertensive, anti-ulcer, calcium channel antagonist, anti-tumor activity and many more activities.

Therefore, based on the previously reported information concerning 1,4.-dihydro pyridine derivatives, I have planned to synthesize some new 1,4dihydro pyridine derivatives with different aldehyde with more biological and chemotherapeutic efficacy as compared to some previously reported dihydro pyridine derivatives with good yield.

So an attempt was made to synthesize dihydro pyridine derivatives and prove their anti-oxidant and anti-microbial activities. The current study contains the following

- Synthesis of 1,4-dihydropyridine derivatives.
- Synthesis of mannich base of 1, 4- dihydropyridine derivatives.
- Characterization of synthesised compounds.
- Screening for Biological Activity

    a) Anti-oxidant activity

    b) Anti-microbial

PLAN OF WORK

Synthesis of the 1,4- dihydro pyridine was carried out in following steps

Step.1

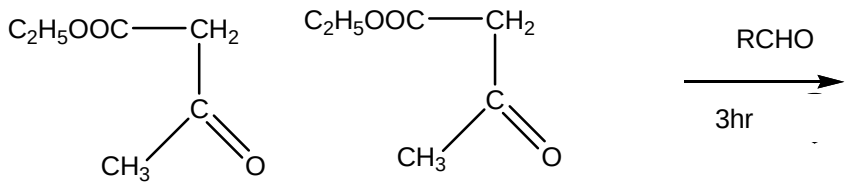
- Synthesis of 1,4-dihydropyridine derivatives.

Step.2

- Synthesis of mannich base of 1,4- dihydropyridine derivatives.

SCHEME OF THE WORK

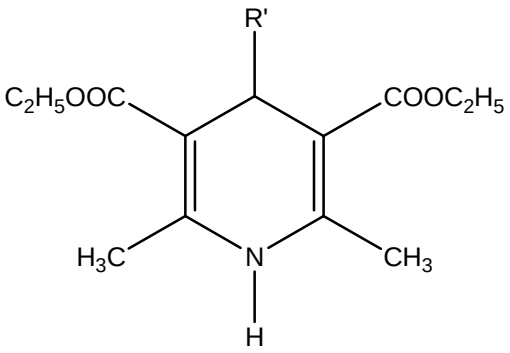
Step 1



Ethyl aceto acetate

\+

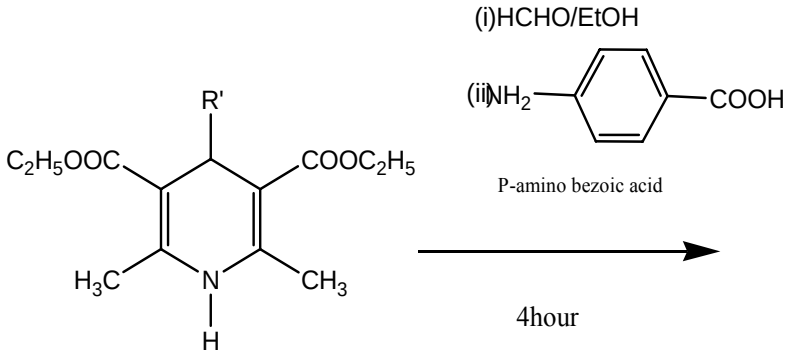
concNH$_4$OH

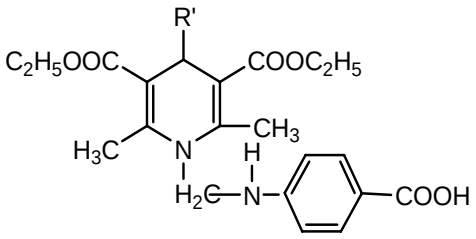


R=C_8H_7 , 3,4-OCH_3-C_6H_4, 2-4-Cl-C_6H_4,

4-CH_3-C_6H_4, 2-OCH_3-4-OH-5-OCH_3-C_6H_2

Step 2





Mannich base of 1,4-dihydropyridine derivatives

R=C_8H_7 , 3,4-OCH_3-C_6H_4, 2-4-Cl-C_6H_4,

4-CH_3-C_6H_4, 2-OCH_3-4-OH-5-OCH_3-C_6H_2

EXPERIMENTAL WORK

Table 1

LIST OF CHEMICALS

S. No.	CHEMICALS USED	MANUFACTURES
1	Ethyl aceto acetate	Chemlabs
2	Ethanol	Chemlabs
3	Ammonia	Chemlabs
4	P-Tolualdehyde	Himedia
5	Cinnamaldehyde	Himedia
6	2,4-dichloro benzaldehyde	Himedia
7	Veratraldehyde	Himedia
8	Syringaldehyde	Himedia

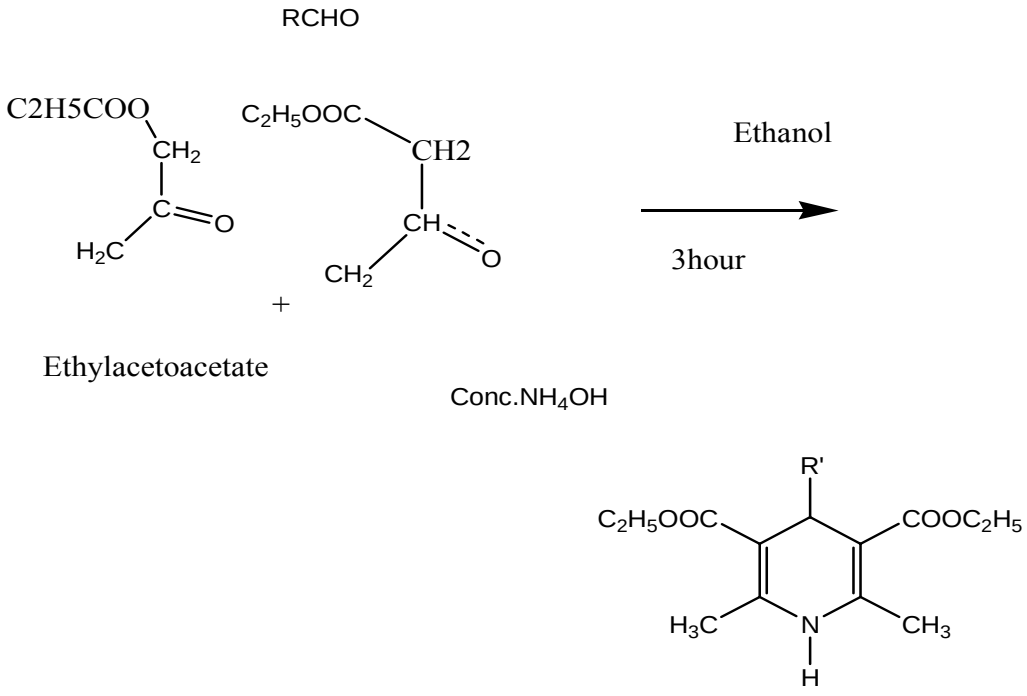
Apparatus; Beaker, conical flask, round bottom flask, condenser made of Borosil glass, funnel, vaccum filter, magnetic stirrer.

PROCEDURE

Step I Preparation of the 1,4-dihydropyridine derivatives

General procedure

A mixture of aldehyde (0.2mole), ethyl acetoacetate (0.2mole) and concentrated ammonium hydroxide (8 ml) in ethanol (60ml) was heated under reflux for 3 hours. To the resulting mixture, warm water (40 ml) was added and then allowed to cool. The separated product was filtered off, washed with 60% aqueous ethanol and recrystallized from alcohol to give Product and it is used for the further reaction **(compound 1a).**



Where,

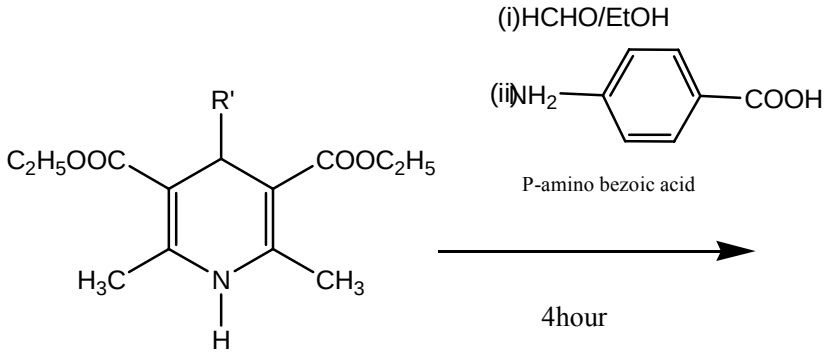
R=C_8H_7, 3,4-OCH_3-C_6H_4, 2-4-Cl-C_6H_4,

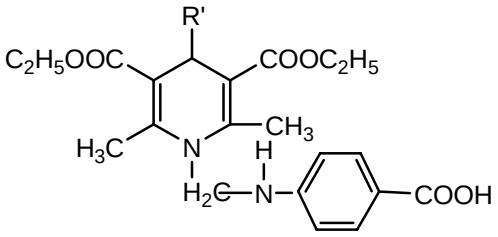
4-CH_3-C_6H_4, 2-OCH_3-4-OH-5-OCH_3-C_6H_2

Similarly, compounds **(1a-e)** were prepared by condensation of ethylacetoacetate and ammonium hydroxide with other aromatic aldehyde.

Step II Preparation of 1,4-dihydropyridine derivatives

A mixture of compound Ia, p-aminobenzoic acid (0.01 mole) and p-formaldehyde (0.02 mole) was taken in 15 ml of rectified spirit and heated under reflux for 4 hours. The reaction mixture was poured on to crushed ice. The product was filtered and recrystallized from aqueous ethanol to give product **(compound 2a).**





Mannich base of 1,4-dihydropyridine derivatives

Where,

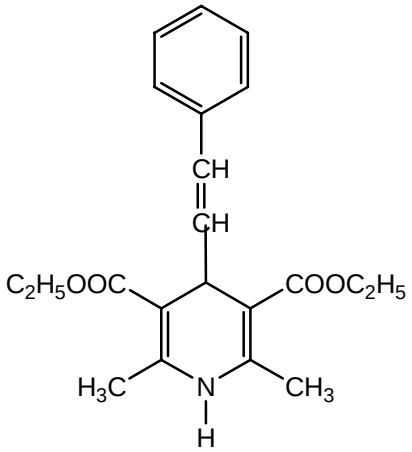
R=C_8H_7 , 3,4-OCH_3-C_6H_4, 2-4-Cl-C_6H_4,

4-CH_3-C_6H_4, 2-OCH_3-4-OH-5-OCH_3-C_6H_2

Similarly, compounds **(2a-e)** were prepared by condensation of p-aminobenzoic acid and p-formaldehyde with product (1a-e).

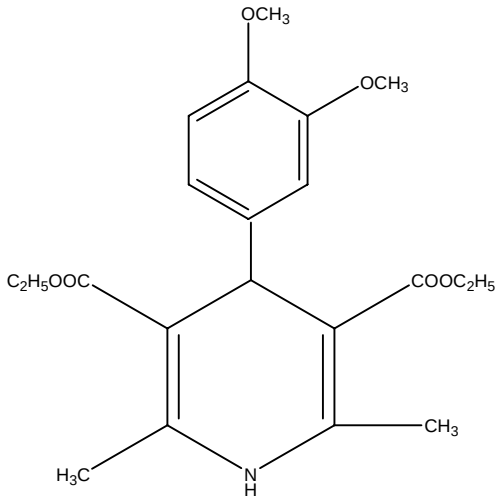
SYNTHESISED DERIVATIVES

1a



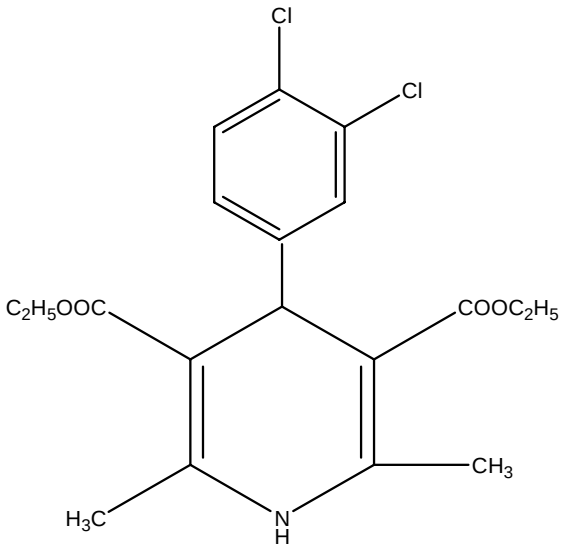
Diethyl2,6-dimethyl-4-styryl-1,4-dihydro pyridine-3,5-dicarboxylate

1b



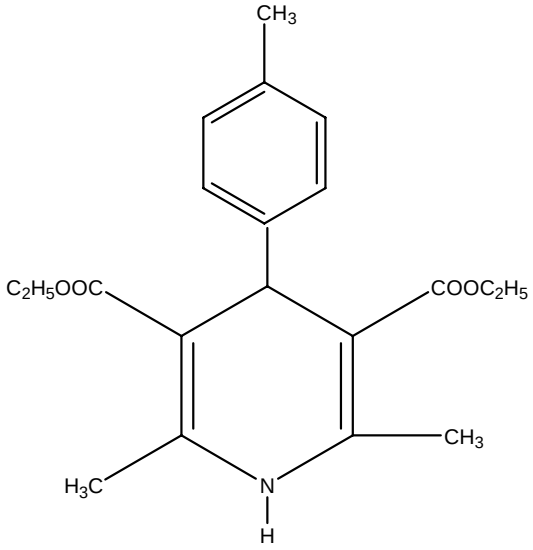
Diethyl4-(3,4-dimethoxyphenyl)2, 6-dimethyl- 1,4-dihydropyridine3,5- dicarboxylate.

1c



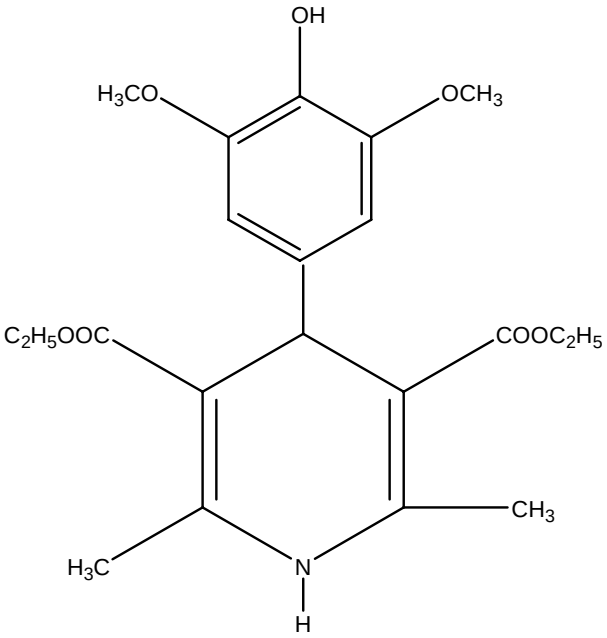
Diethyl4-(2,4-dichloro phenyl)- 2,6-dimethyl- 1, 4 –dihydropyridine-3,5-dicarboxylate.

1d



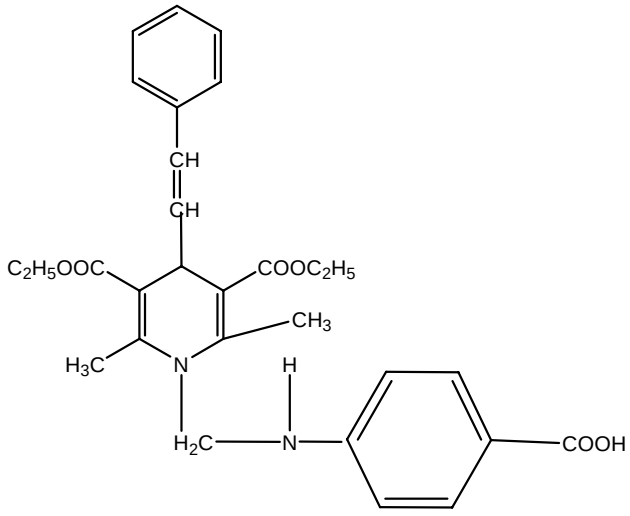
Diethyl 2, 6-dimethyl-4-p-tolyl-1,4-dihydropyridine-3,5-dicarboxylate

1e



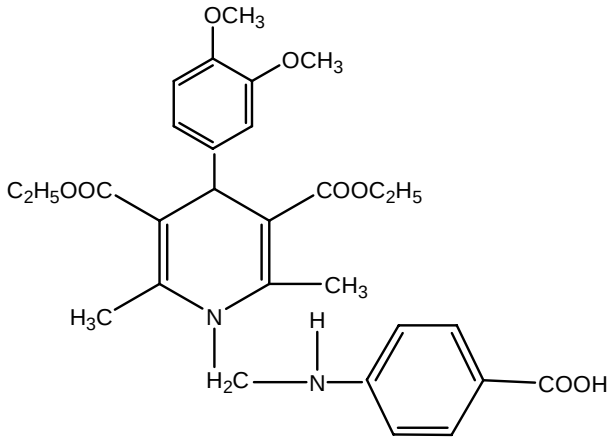
Diethyl4-(4-hydroxy-3, 5-dimethoxy-2-phenyl)-2, 6-dimethyl-1,4dihydro pyridine-3, 5-dicarboxylate

2a



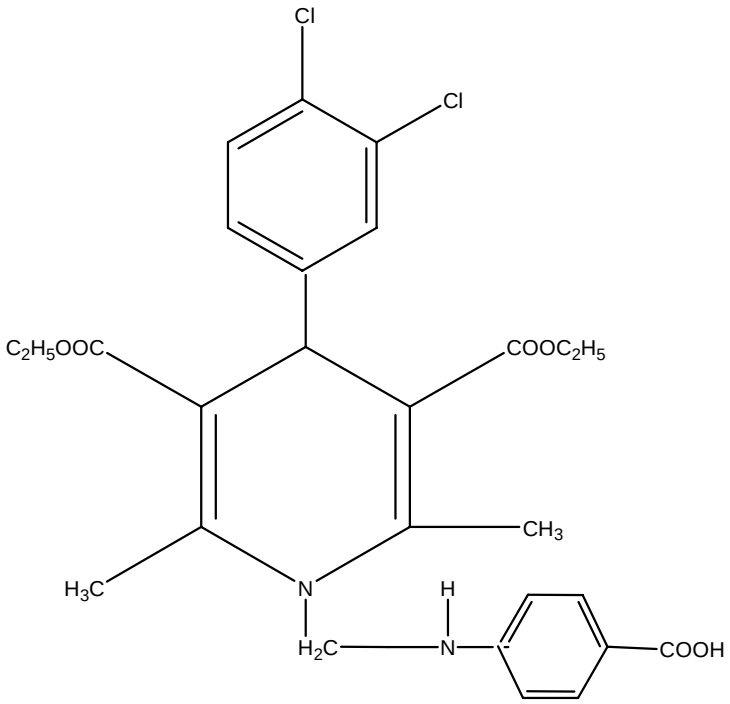
4-((3,5-bis(ethoxycarbonyl)-2,6-dimethyl-4-styrylpyridine-1(4H)-yl)methylamino)benzoic acid.

2b



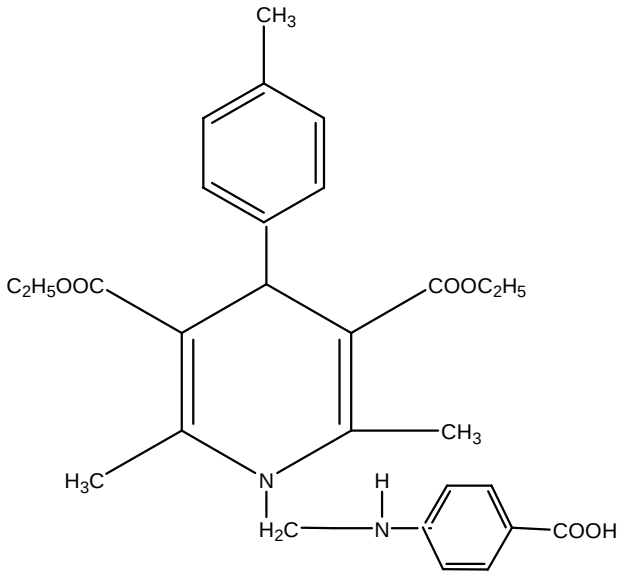
4-((3,5-bis(ethoxycarbonyl)-2,6-dimethyl-4-(3,4-dimethoxy phenyl)pyridine-1(4H)-yl) methylamino) benzoic acid.

2c



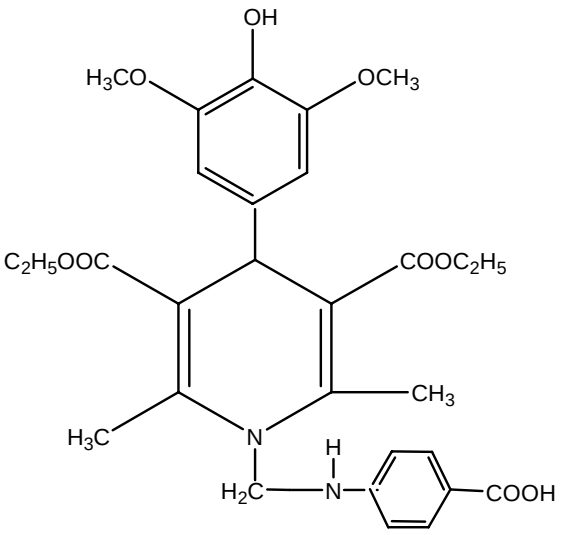
4-((3,5-bis(ethoxycarbonyl)-2,6-dimethyl-4-(3,4dichlorophenyl)pyridine-1-(4H)-yl)methyamino) benzoic acid.

2d



4-((3,5-bis(ethoxycarbonyl)-2,6-dimethyl-4-p-tolylpyridine-1-(4H)-yl) methylamine)benzoic acid.

2e



4-(3,5-bis(ethoxycarbonyl)2,6-dimethyl-4-(4hydroxy3,5dimethoxypheny)pyridine-1 -(4H)-yl) methylamino)benzoic acid.

CHARACTERIZATION

The synthesized compounds were purified by recrystallization and thin layer chromatography. The compounds were then subjected to spectral characterization and elemental analysis.

The melting point was determined by open capillary tube method and it was uncorrected. Analysis was performed in Heraceus CHN Rapid analyzer (division of catalysis and kinetics, Department of Chemistry, **Indian Institute of Technology**, Chennai,). The data is presented in **Table 2**.

IR Spectra was recorded (KBr) on ABB BOMEM FTIR spectrophotometer MB serial II–Canada (Mical Lab, Chennai-32). The data is presented in **Table 4**.

1H NMR Spectrum (DMSO) was recorded on 400 MHZ-Joel DPX (Indian Institute of Technology, Chennai, Tamil Nadu, India) using tetra methyl Silane as internal standard. The data is presented in a **Table 6**.

Mass spectra were recorded on Joel GC mate-II, GCMS system (Sophisticated Analytical Instrument facility, Indian Institute of Technology, Chennai, Tamil Nadu). The data is presented in a **Table 5**.

Table 2

Various substitution and physicochemical property of dihydropyridine derivatives

Compound	R	R_F value	M..P (^oC)	% Yield
1a	C_8H_7	0.69	131-132	65
1b	3,4-OCH_3-C6H_4	0.62	126-128	68
1c	2,4-Cl – C_6H_4	0.71	123-125	67
1d	4-CH_3-C_6H_4	0.23	146-149	70
1e	2-OCH_3-4-OH-5-OCH_3-C_6H_2	0.7	120-125	63
2a	C_8H_7	0.31	123-124	62
2b	3,4-OCH_3-C_6H_4	0.39	131-135	63
2c	2,4-Cl-C_6H_4	0.67	145	62
2d	4-CH_3-C_6H_4	0.71	148-155	65
2e	2-OCH_3-4-OH-5-OCH_3-C_6H_2	0.20	125-134	60

Mobile phase Hexane: Ethyl acetate (2:1)

Table 3

Elemental analytical data of synthesized compounds

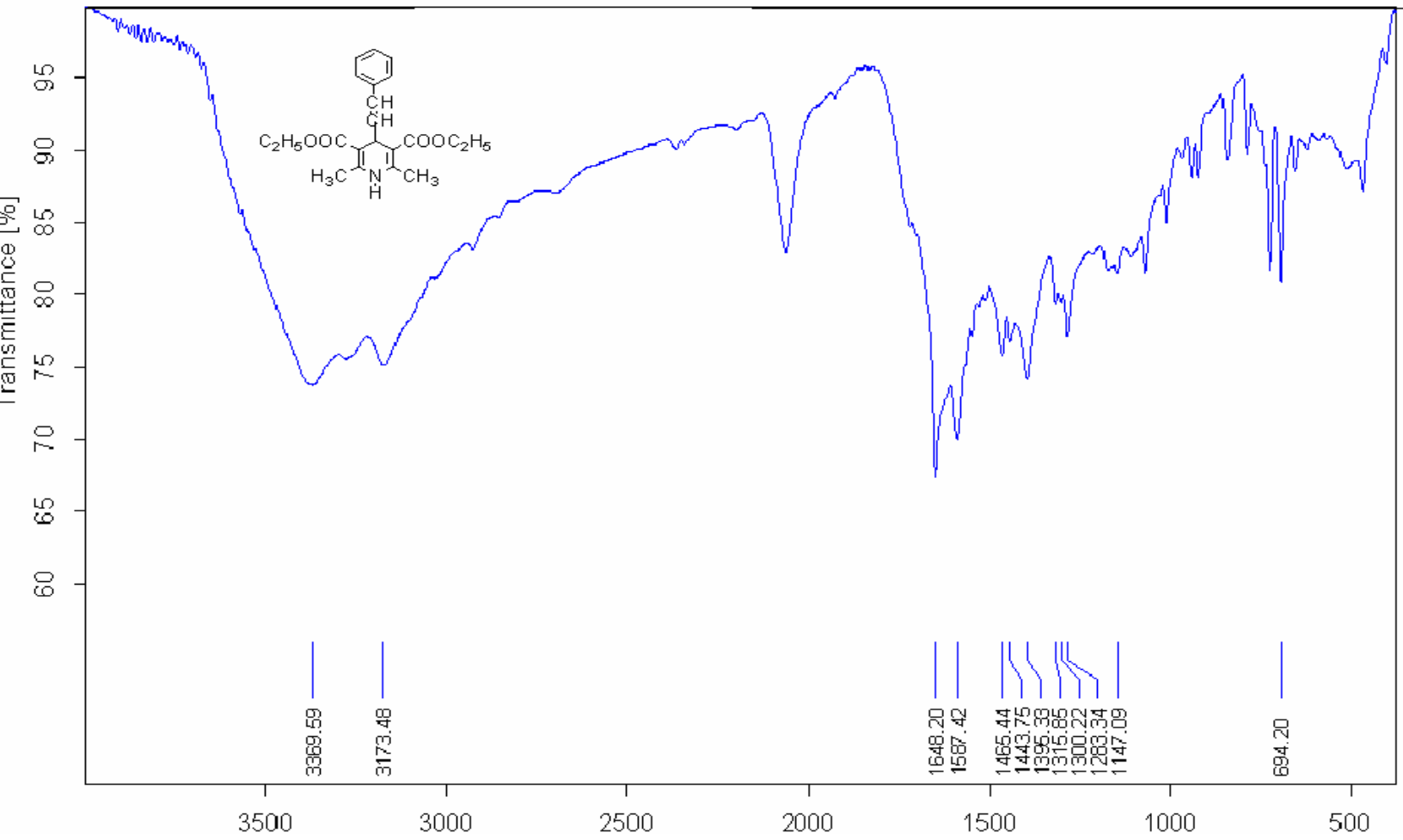
Compound	Mol. Formula	Molecular Weight	Yield in percentage	Elemental analysis Calculated (Experimental)				
				C	H	N	O	
1a	$C_{29}H_{32}N_2O_6$	355.43	65	70.96	7.09	3.94	18.01	
1b	$C_{28}H_{32}N_2O_7$	389.44	68	64.16	5.83	3.86	17.64	9
1c	$C_{27}H_{28}N_2O_6Cl_2$	361.64	67	62.90	5.83	3.86	17.64	9
1d	$C_{28}H_{32}N_2O_6$	343.42	70	69.95	6.72	3.45	27.62	
1e	$C_{29}H_{34}N_2O_9$	450.16	63	62.21	6.71	3.45	27.62	
2a	$C_{29}H_{32}N_2O_6$	504.57	62	69.03	6.39	5.55	19.03	
2b	$C_{28}H_{32}N_2O_7$	508	63	64.67	6.36	5.29	23.76	
2c	$C_{27}H_{28}Cl_2N_2O_6$	510.70	62	59.24	5.16	5.12	17.54	1
2d	$C_{28}H_{32}N_2O_6$	492	65	68.28	6.55	5.69	19.49	
2e	$C_{29}H_{34}N_2O_9$	554	60	62.81	6.18	5.05	25.96	

Table 4

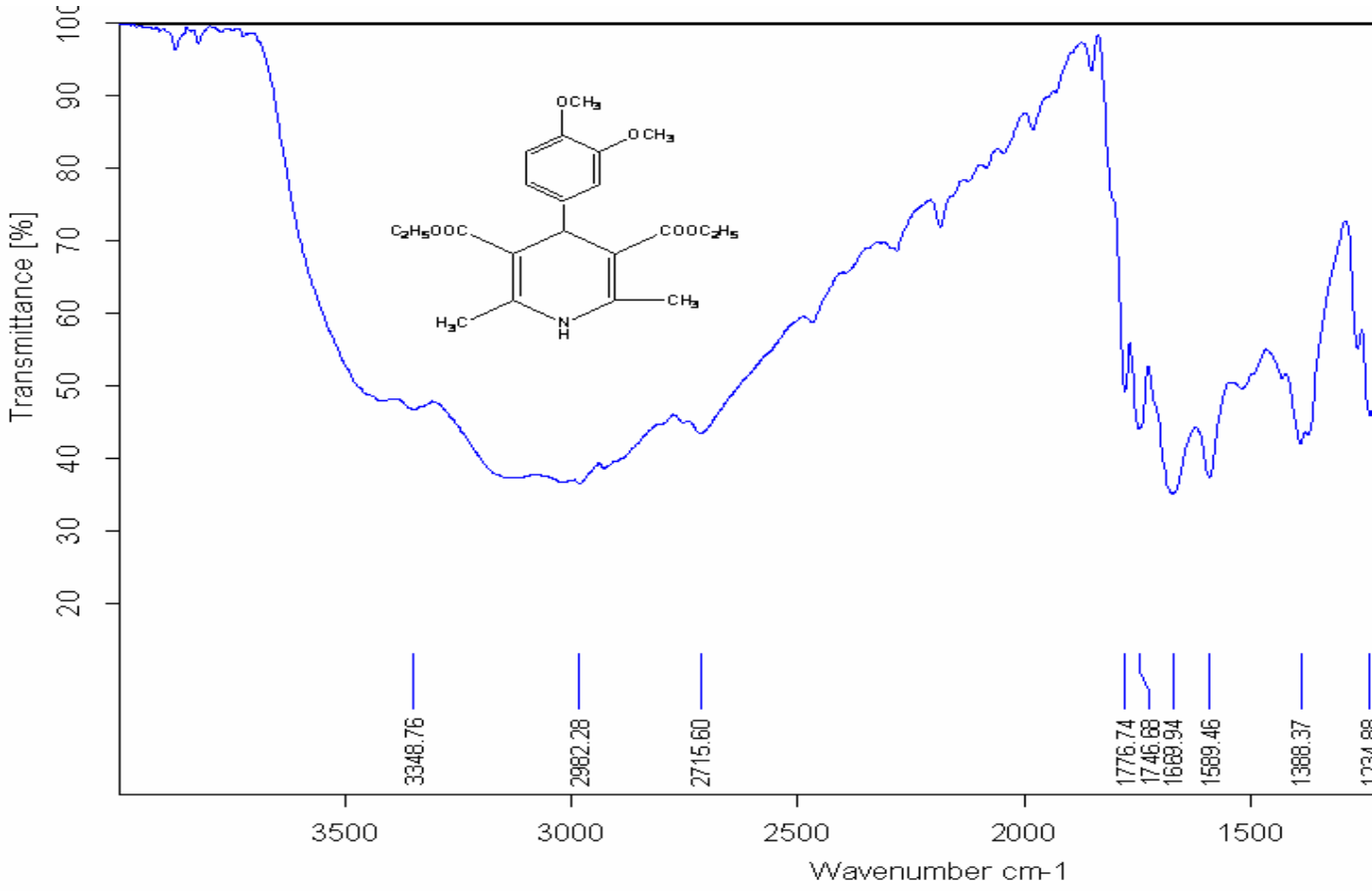
IR spectra of the compounds

S.No.	COMPOUNDS	WAVE NUMBER (cm^{-1})
1	1a	3369,3173,1648,1587,1465,1443,1396,1315,1300, 1283,1147,1069,724,694.
2	1b	3348,2982,2715,1776,1746,1669,1589,1388,1234,1198,712,518,478.
3	1c	3380,2696,2062,1621,1466,1400,1269,693,505.
4	1d	3409,3199,1649,1581,1444,1393,1315,1298,1282,1067,842,724,694.
5	1e	3667,3643,3624,3606,3582,3541,3497,3457,3440,3026,1744,1728,1712,1694,168 1536, 1445,1393,1283.
6	2a	3177,2053,1776,1665,1590,1509,1399,1371,1263,1231,1200,713,673,476.
7	2b	3333,1651,1605,1580,1511,1475,1443,1360,1280,1217,1166,567.
8	2c	3464,3172,3062,2976,1721,1662,1579,1530,1447,1346,1234,1150,928,830,735,5
9	2d	3365,3267,3166,2061,1647,1587,1466,1444,1393,1299,1283,1068,724,693.
10	2e	3369,3173,1648,1582,146,1443,1396,1315,1300,1283,1147,694.

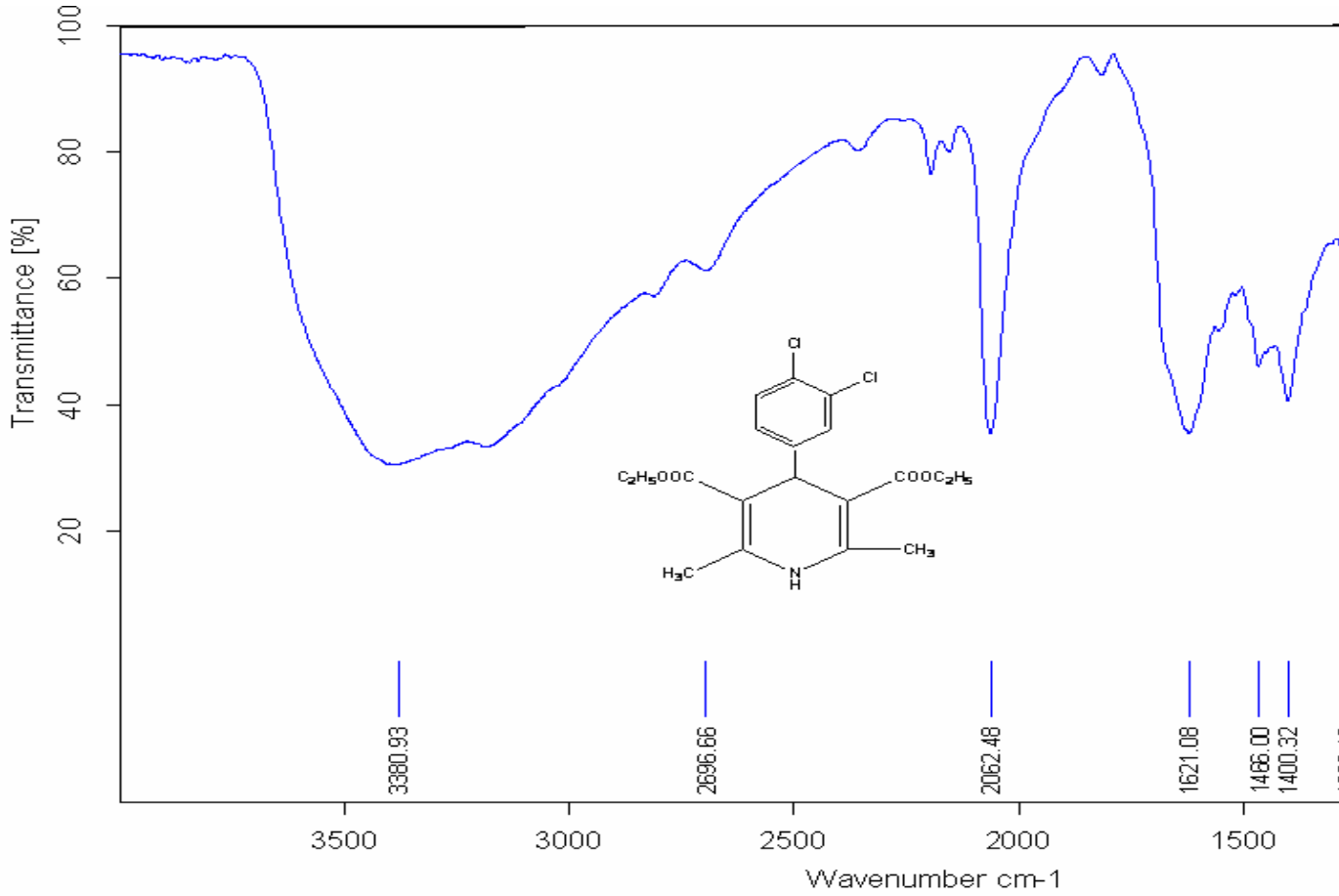
Fig,1 IR Spectra of compound-1a



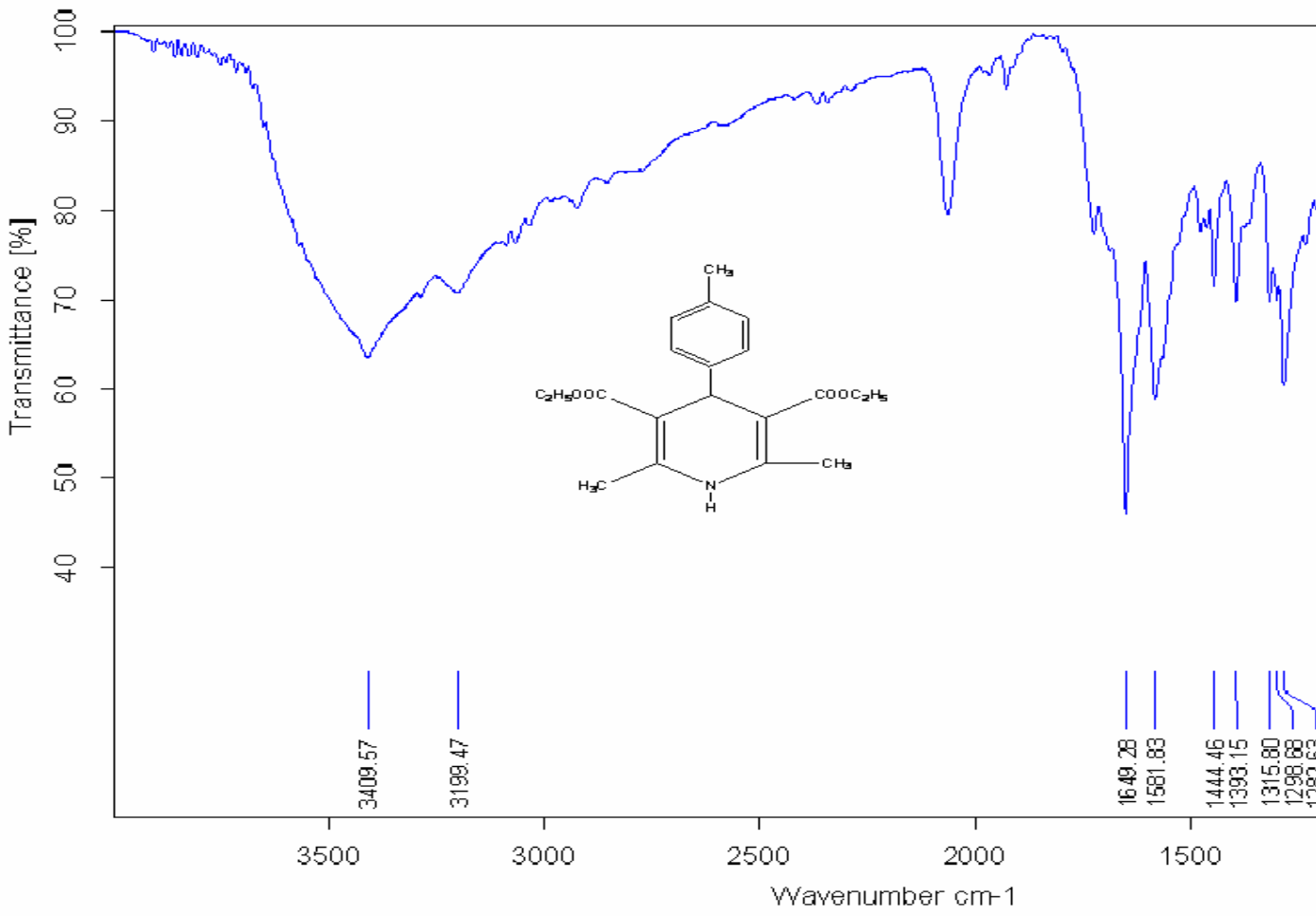
Fig,2 IR Spectra of compound-1b



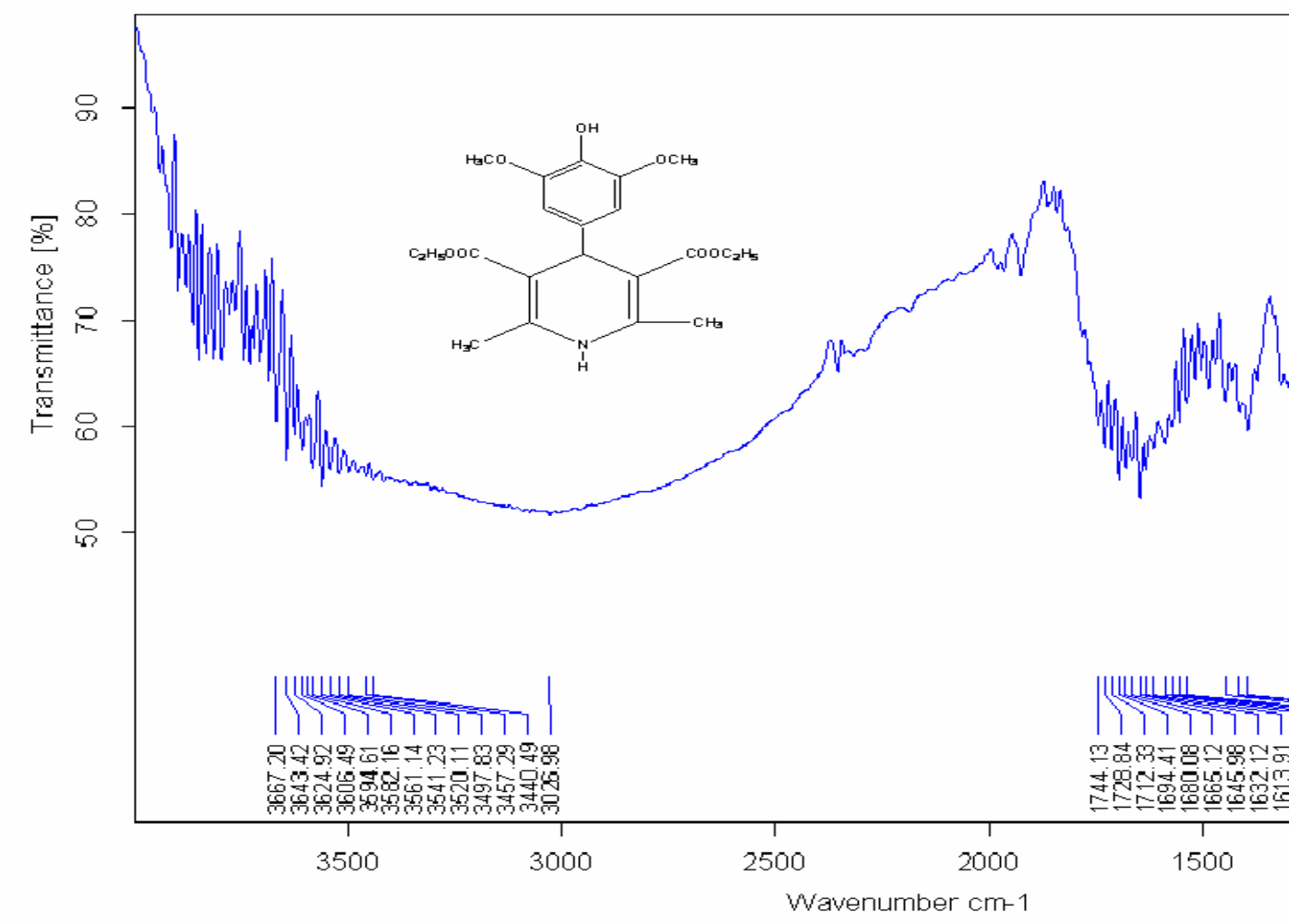
Fig,3 IR Spectra of compound-1c



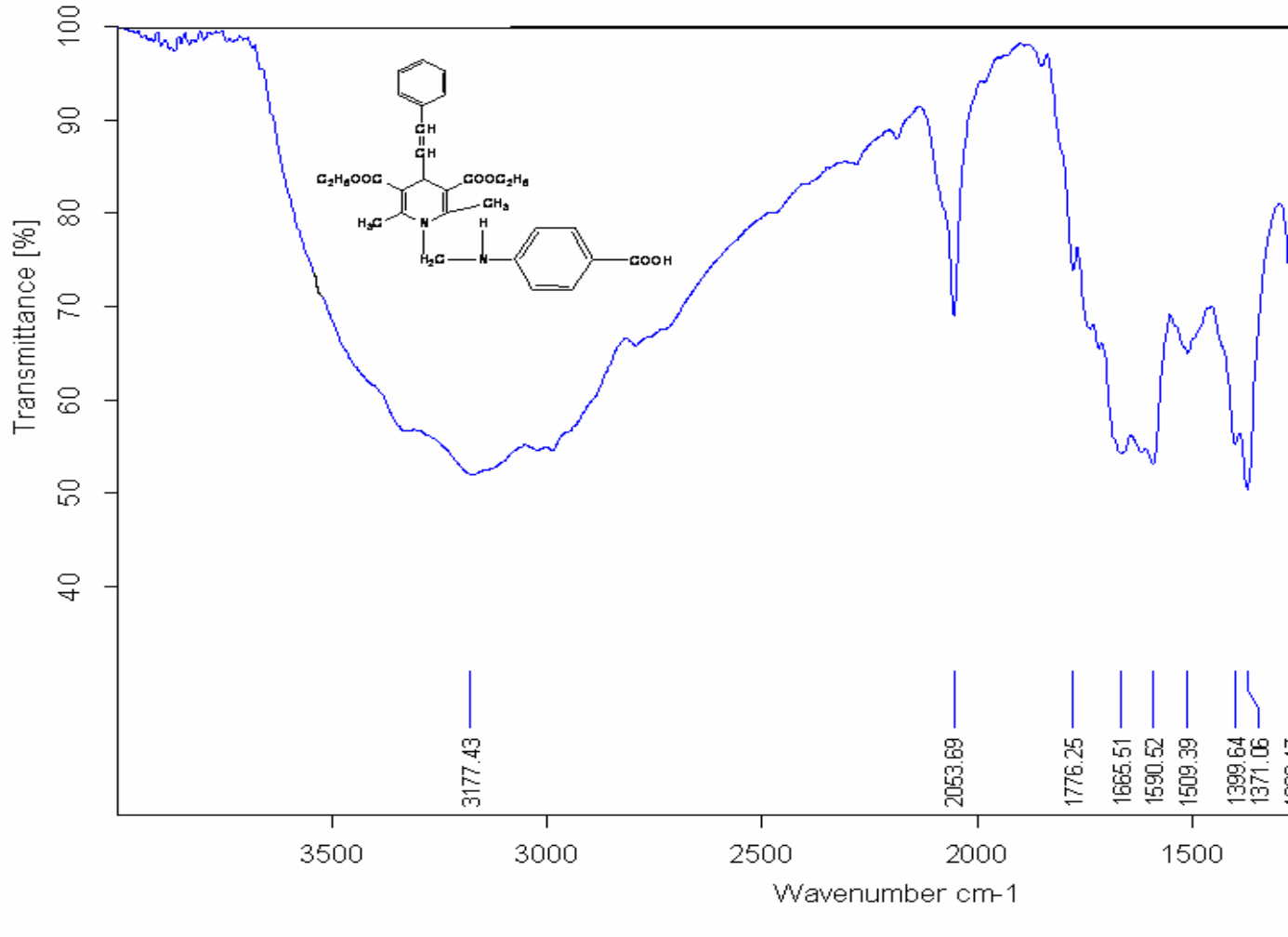
Fig,4 IR Spectra of compound-1d



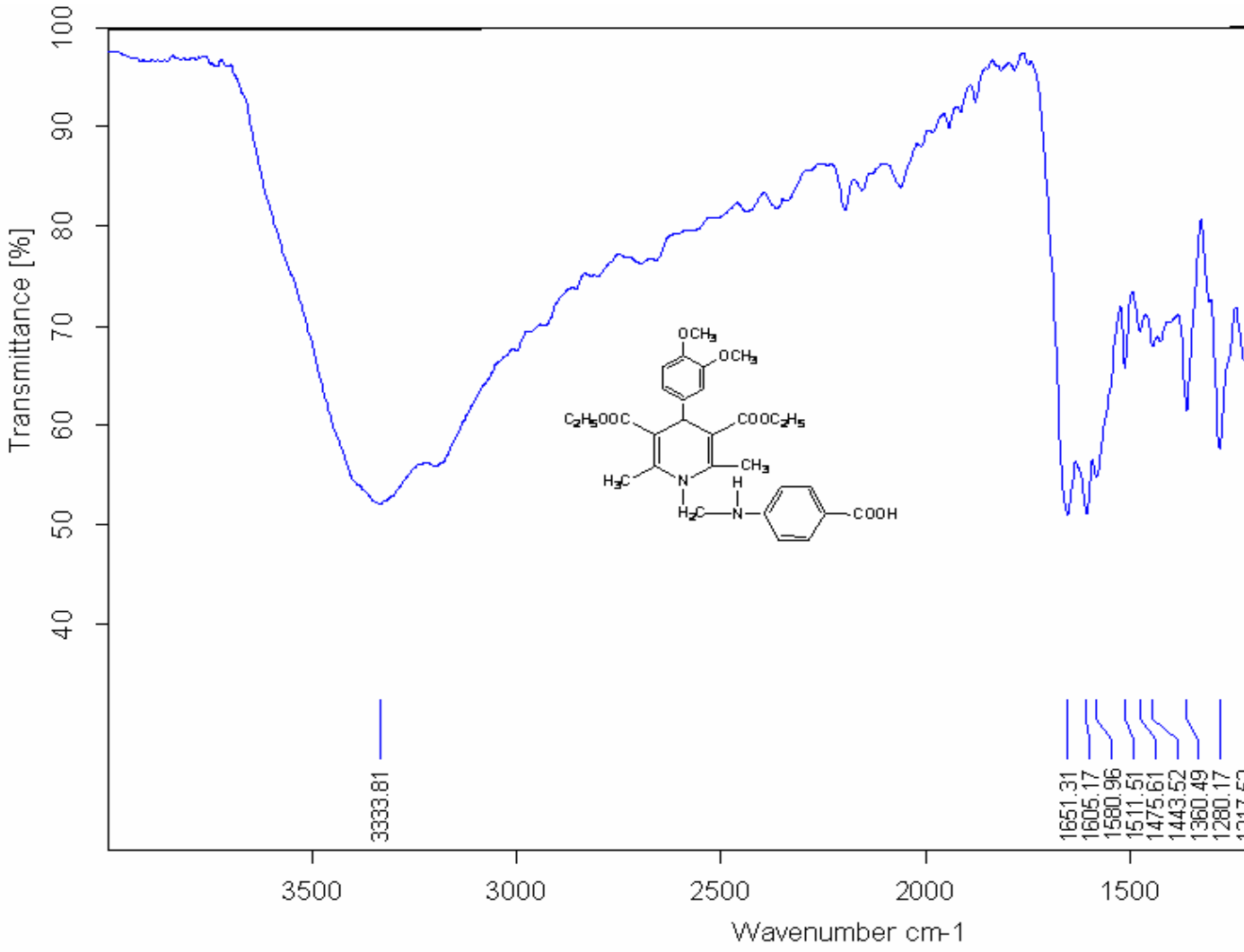
Fig,5 IR Spectra of compound-1e



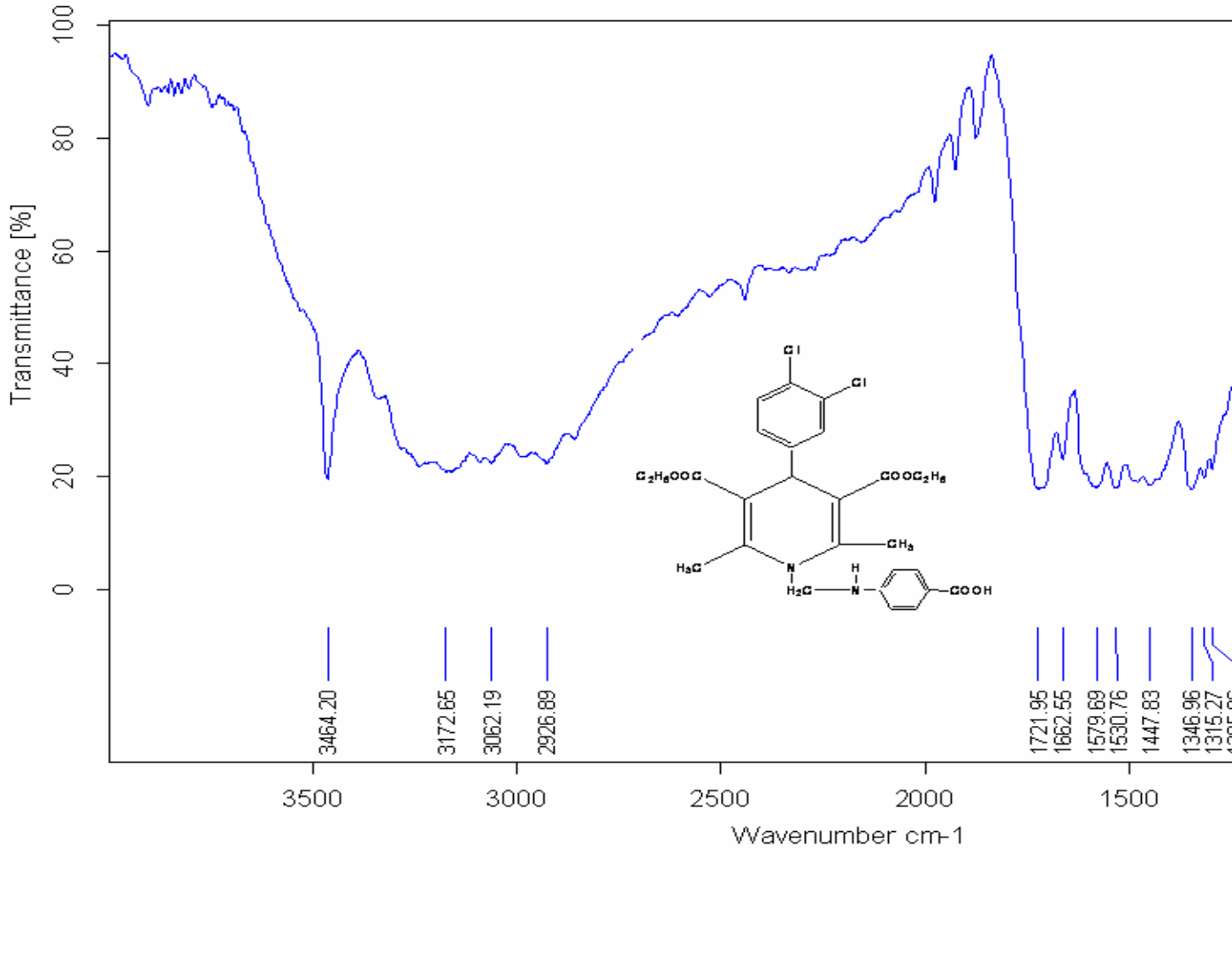
Fig,6 IR Spectra of compound-2a



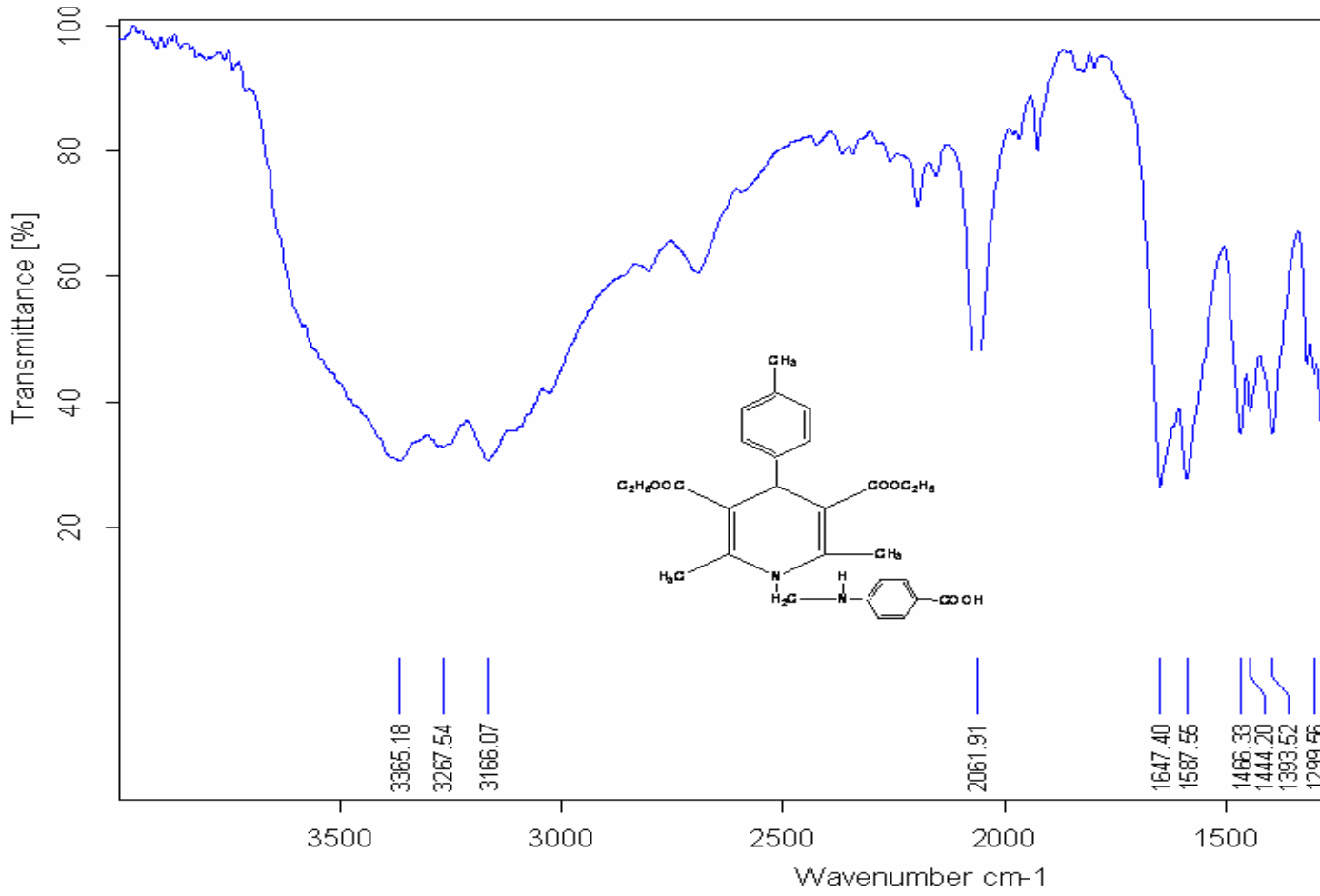
Fig,7 IR Spectra of compound-2b



Fig,8 IR Spectra of compound-2c



Fig,9 IR Spectra of compound-2d



Fig,10 IR Spectra of compound-2e

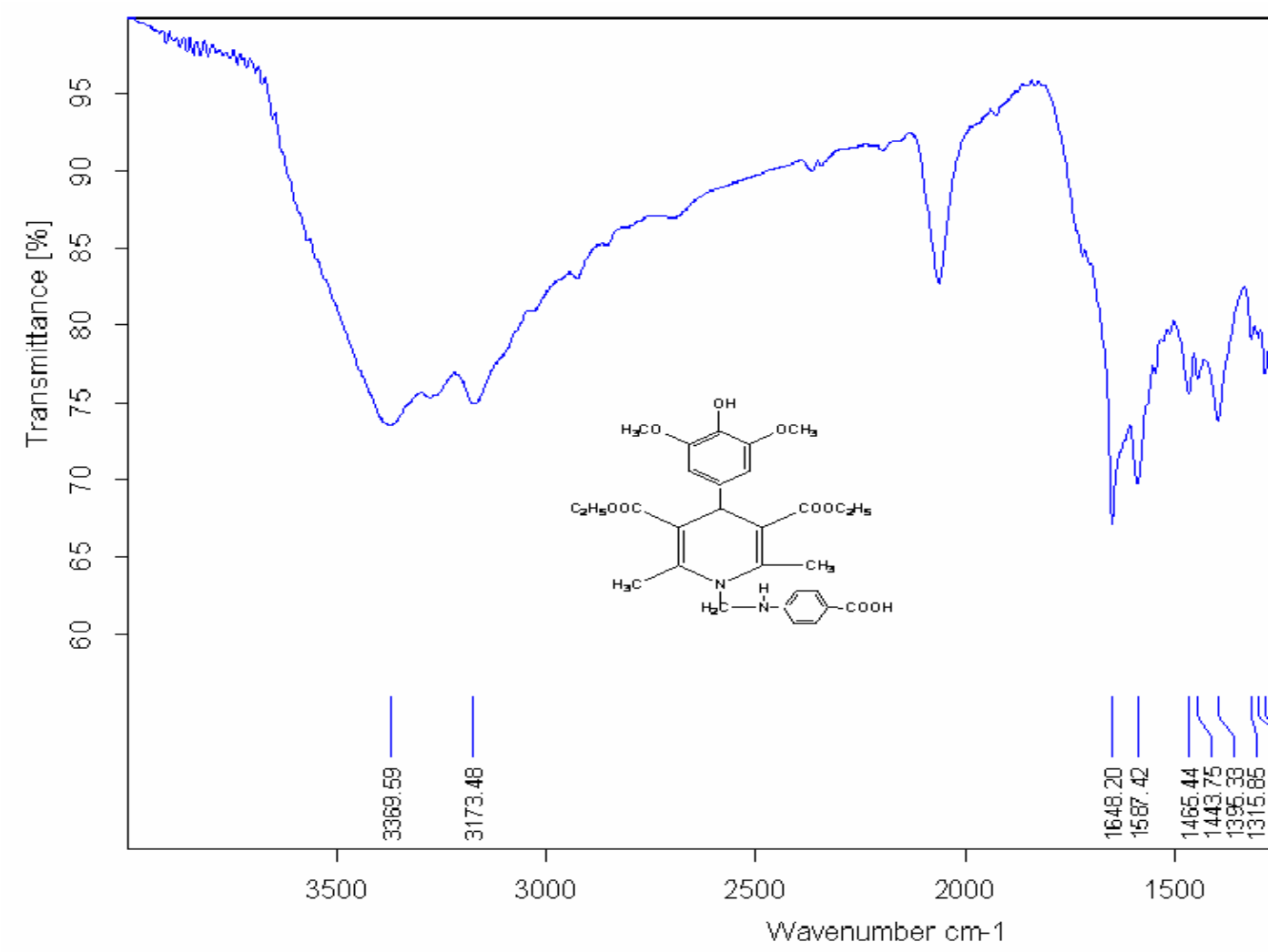

Transmittance [%]
95
90
85
80
75
70
65
60
3369.59
3173.48
1648.20
1587.42
1465.44
1443.75
1395.33
1315.85
3500
3000
2500
2000
1500
Wavenumber cm-1
OH
H3CO
OCH3
C2H5OOC
COOC2H5
CH3
H3C
N
H
H2C
N
COOH

Table 5

MASS spectral data of the synthesized compounds

S.No.	Compounds	Molecular Weight	M/Z (%Relative Abundance)
1	**1a**	355.43	355.58 M^+ (5%), 353.55(10%), 340.51(4%), 326.52(6%), 308.58(5% 252.50(25%), 236.49(8%), 215.44(6%), 206.45(7%). 196.43(8%),
2	**1b**	389.44	387.66M^+(57%), 278.52(5%), 263.50(6%), 252.51(5%) ,1 95.35(50%),77.32(38%),65.31(18%),
3	**1c**	361.64	362.50M^+(5%), 286.60(20%), 270.63(5%), 251.46(100%), 205.40(40%), 175.29(40%), 58.29(10%).
4	**1d**	343.42	343.65M^+(5%), 312.62(6%), 270.60(18%), 252.57(99%), 145.42(20%).\, 119.40(38%), 65.33(18%),
5	**1e**	450.16	450.65M^+(3%), 438.49(4%), 376.64(4%), 332.57(8%), 314.69(5% 252.57(20%), 224.49(15%), 196.45(18%), 77.32(5%).
6	**2a**	504.57	504.92M^+(3%),467.70(3%),252.51(58%),236.51(18%),196.43(20%) 61.29(50%),
7	**2b**	508	508.37M^+(18%), 475.30(20%), 455.53(18%), 398.58(16% 316.60(18%),264.46(12%),252.57(55%), 149.39(25%),137.37(55%)
8	**2c**	510.70	511.37M^+(7%),483.07(7%),358.13(7%),299.56(5%),251.46(32%),22 205.40(15%),75.36(15%),120.43(8%), 83.36(9%).
9	**2d**	492	492.81M^+(3%), 341.60(8%), 252.54(98%), 224.49(19%), 120.39(70%), 65.32(35%0.
10.	**2e**	554	555.87M^+(%), 527.67(9%), 403.59(18%), 372.22 (18%), 232.57(98%), 224.54(22%), 154.41(22%), 137.42 (52%), 120.40 (90

Fig-11 MASS Spectra of Compound- 1a

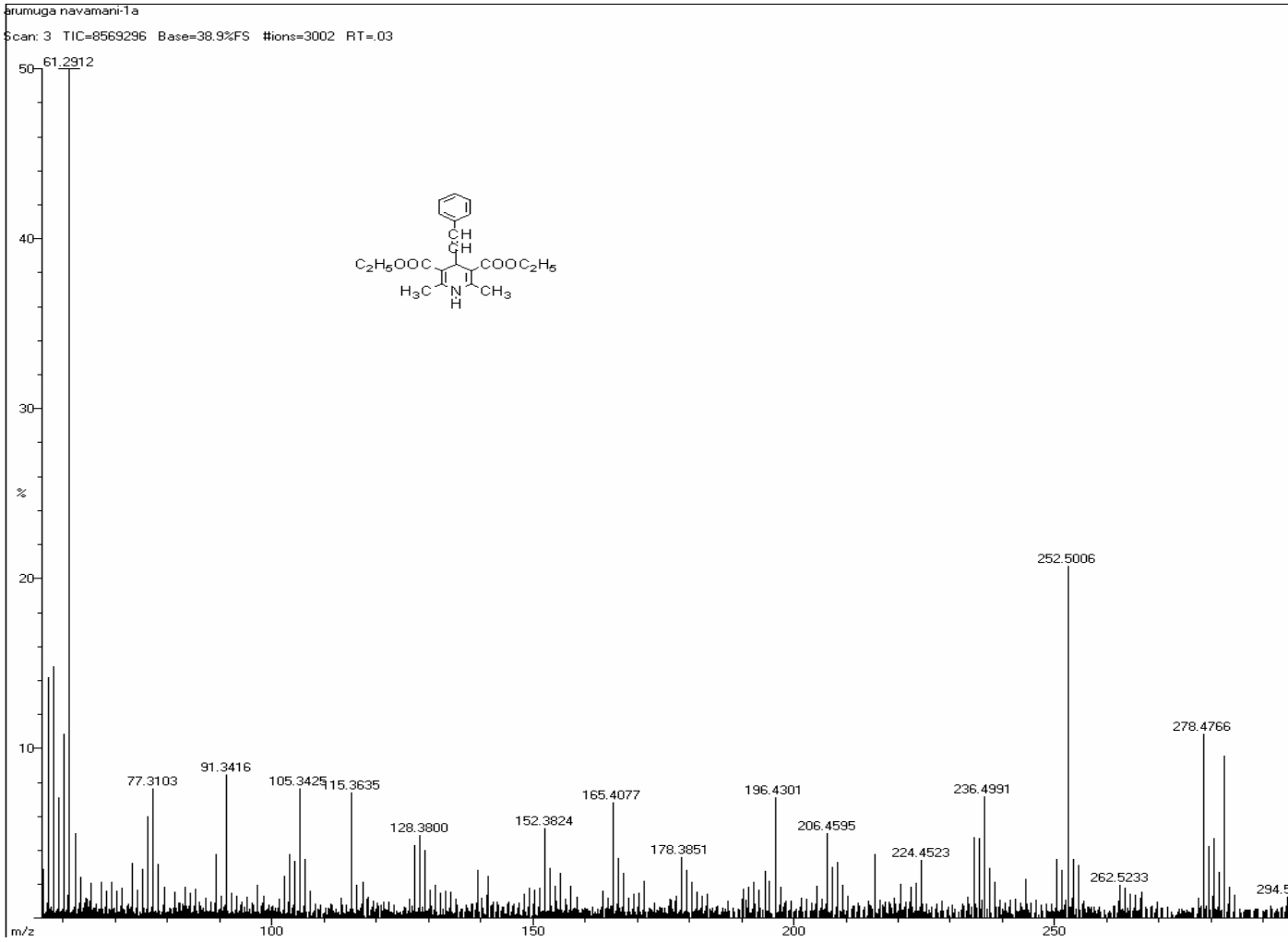


Fig-12 MASS Spectra of Compound- 1b

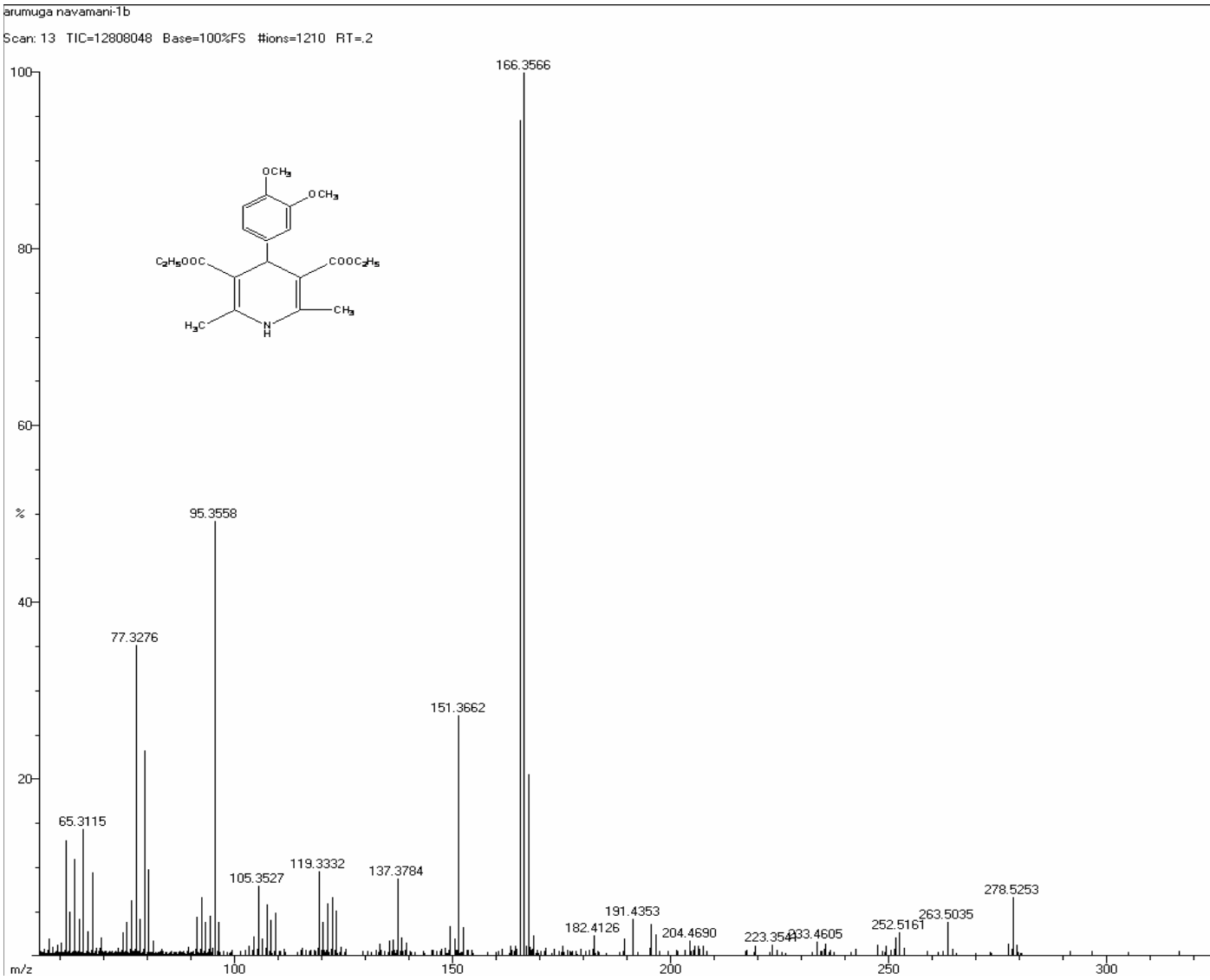


Fig-13 MASS Spectra of Compound- 1c

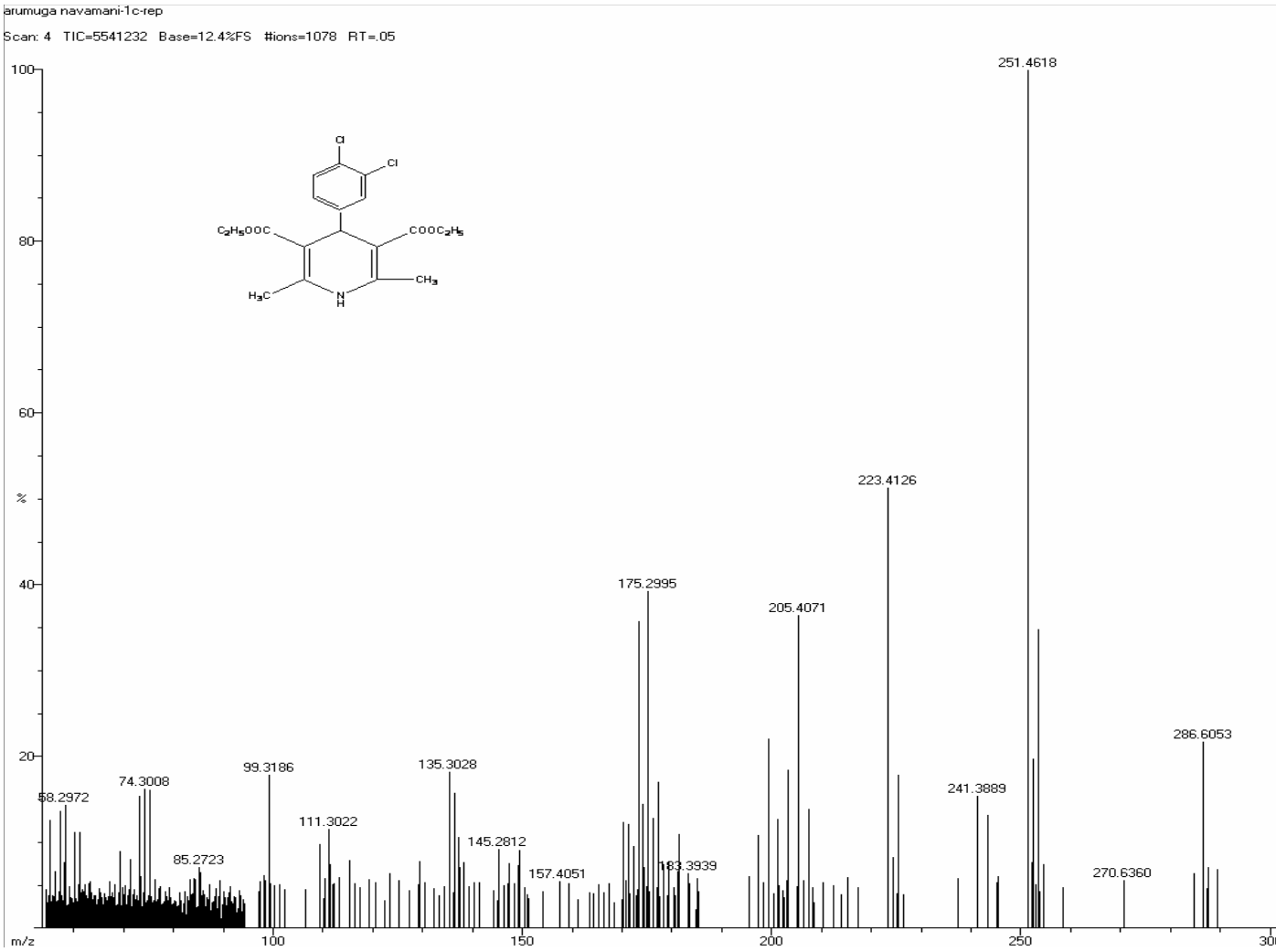


Fig-14 MASS Spectra of Compound- 1d

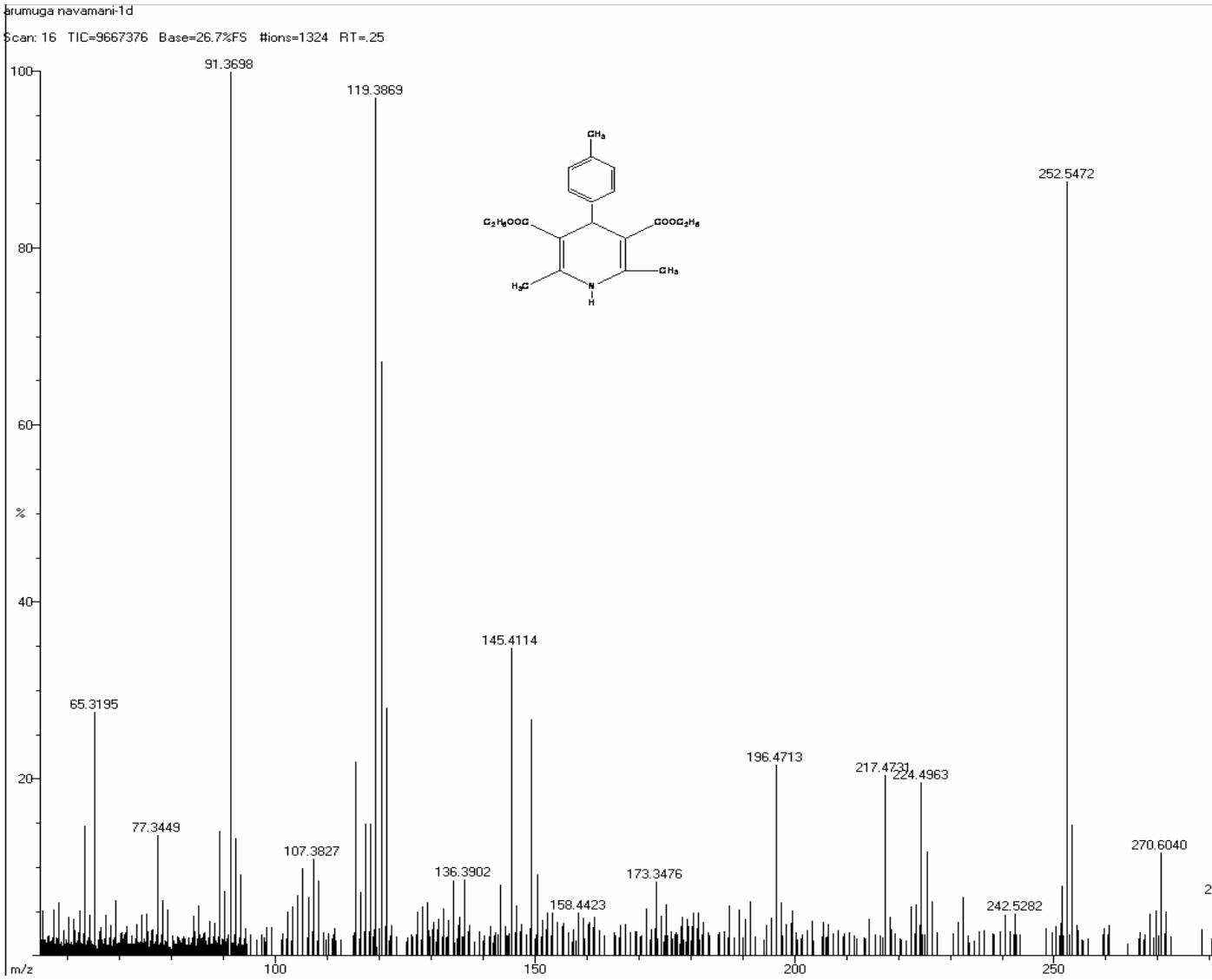


Fig-15 MASS Spectra of Compound- 1e

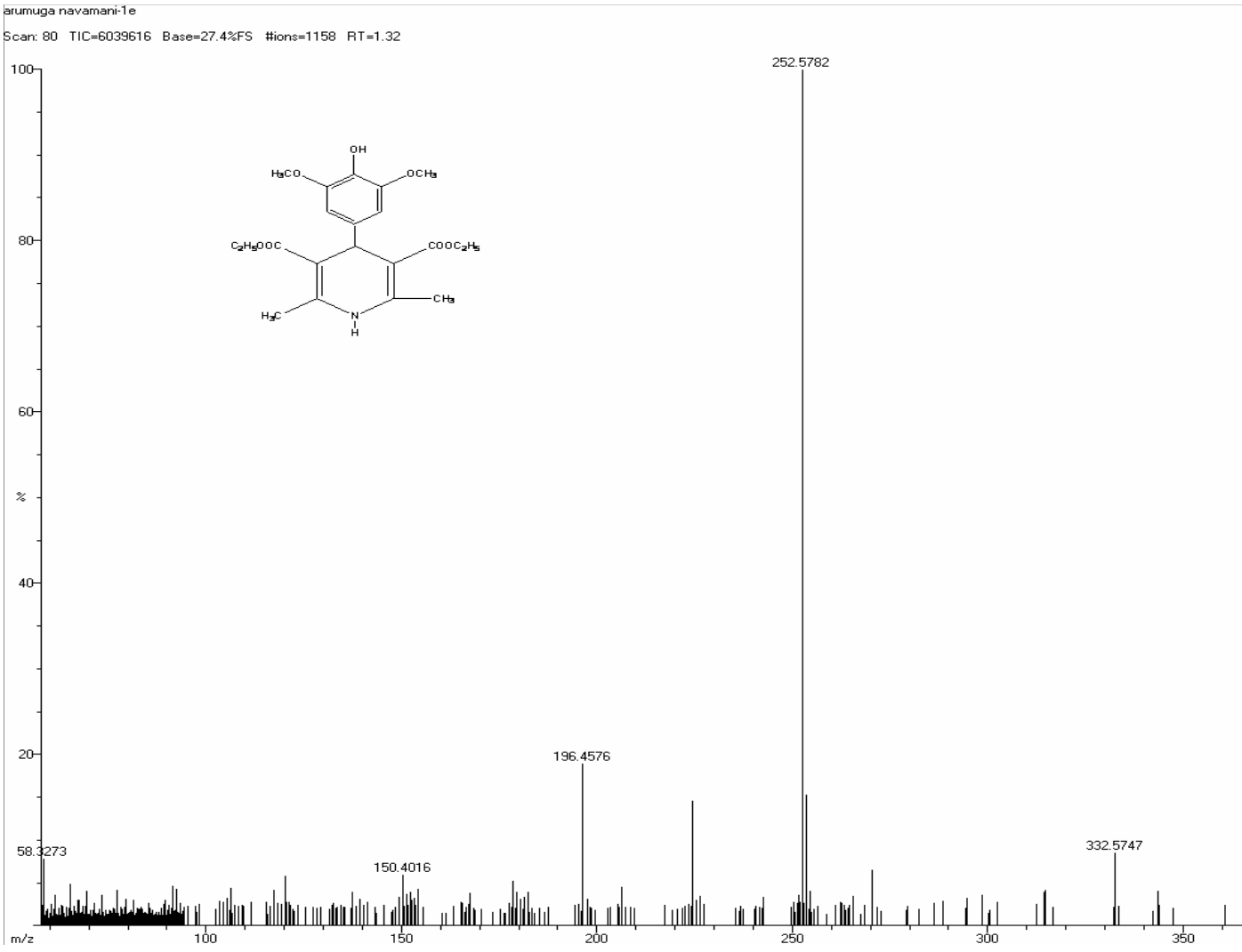


Fig-16 MASS Spectra of Compound- 2a

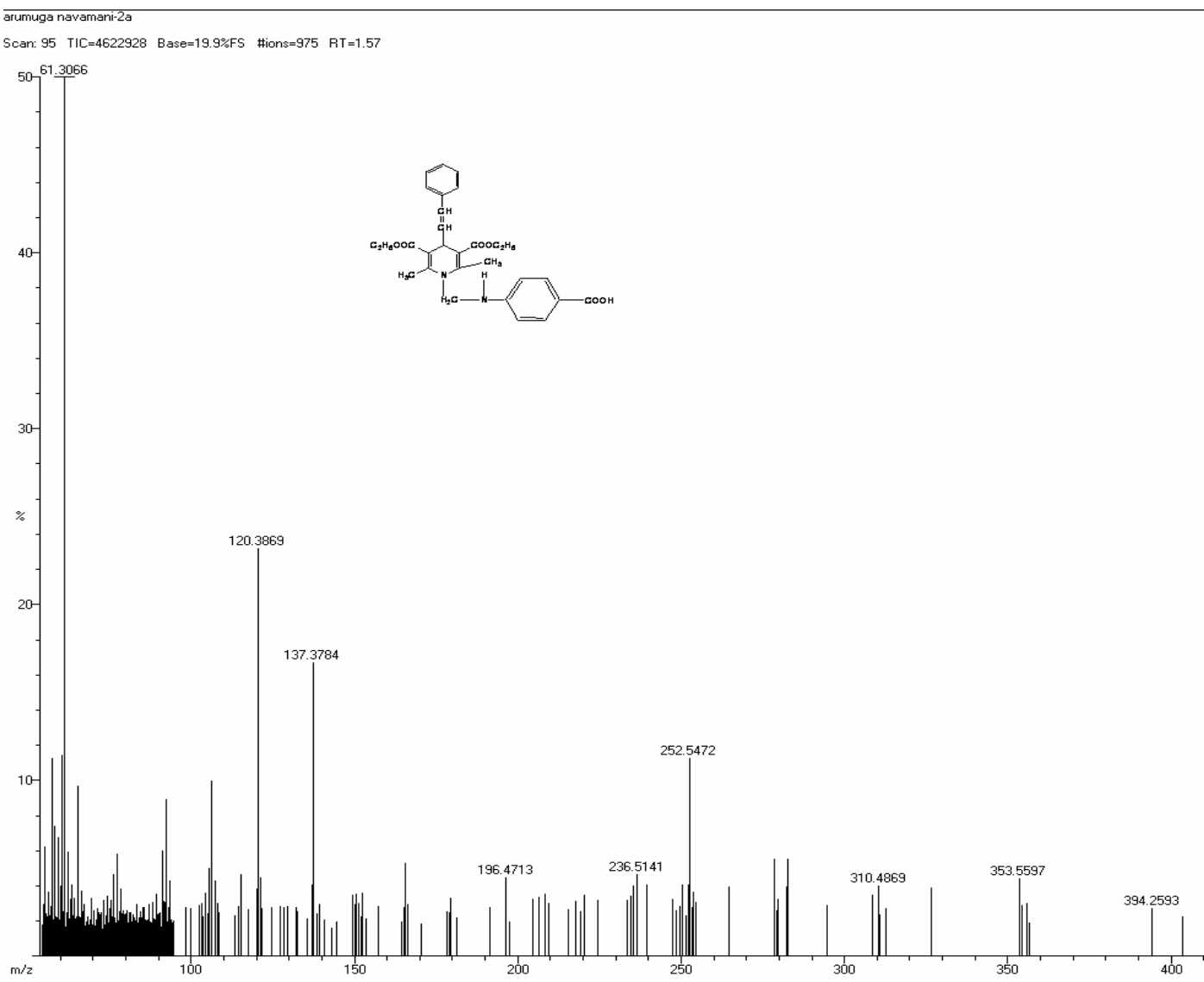


Fig-17 MASS Spectra of Compound- 2b

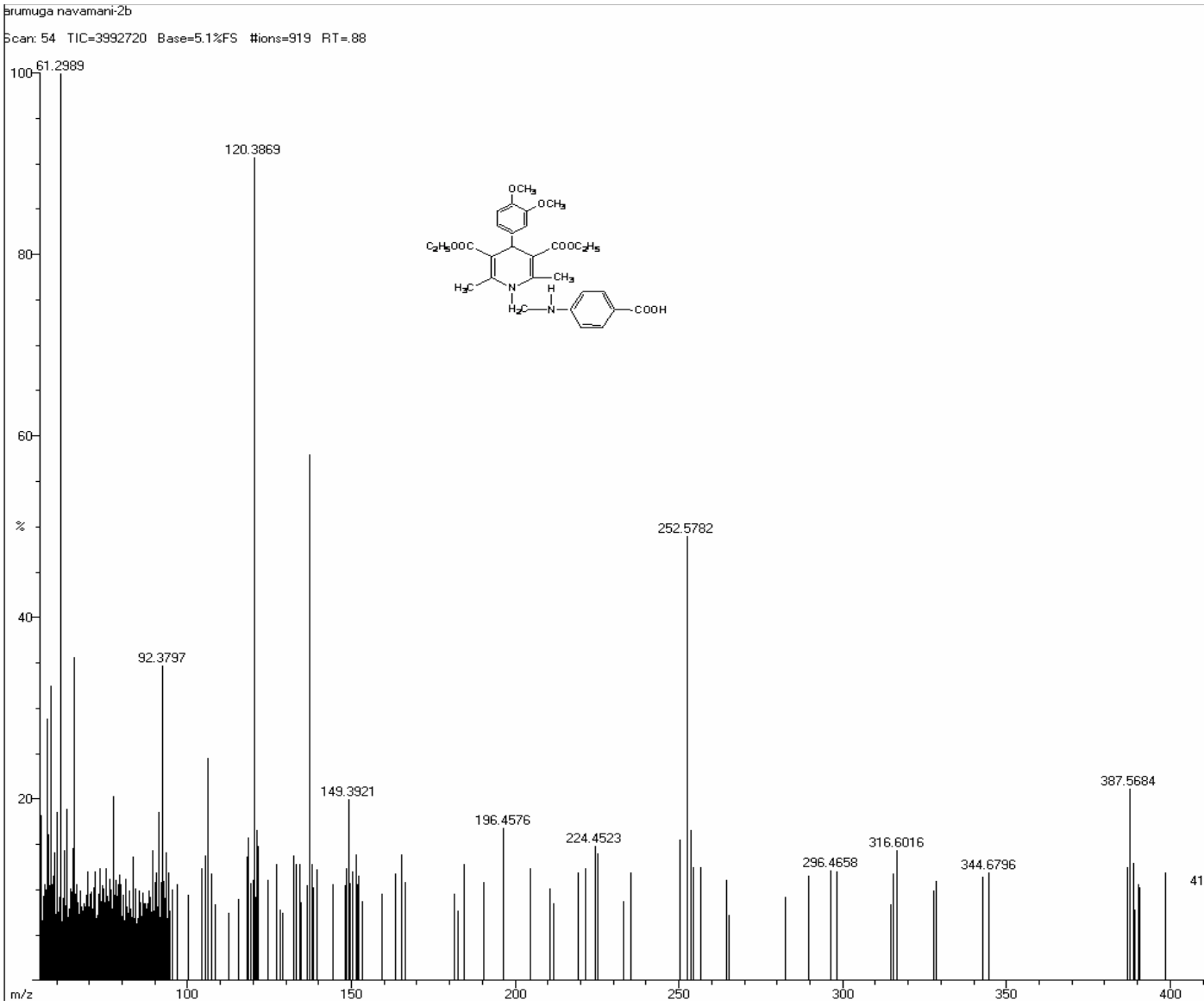


Fig-18 MASS Spectra of Compound- 2c

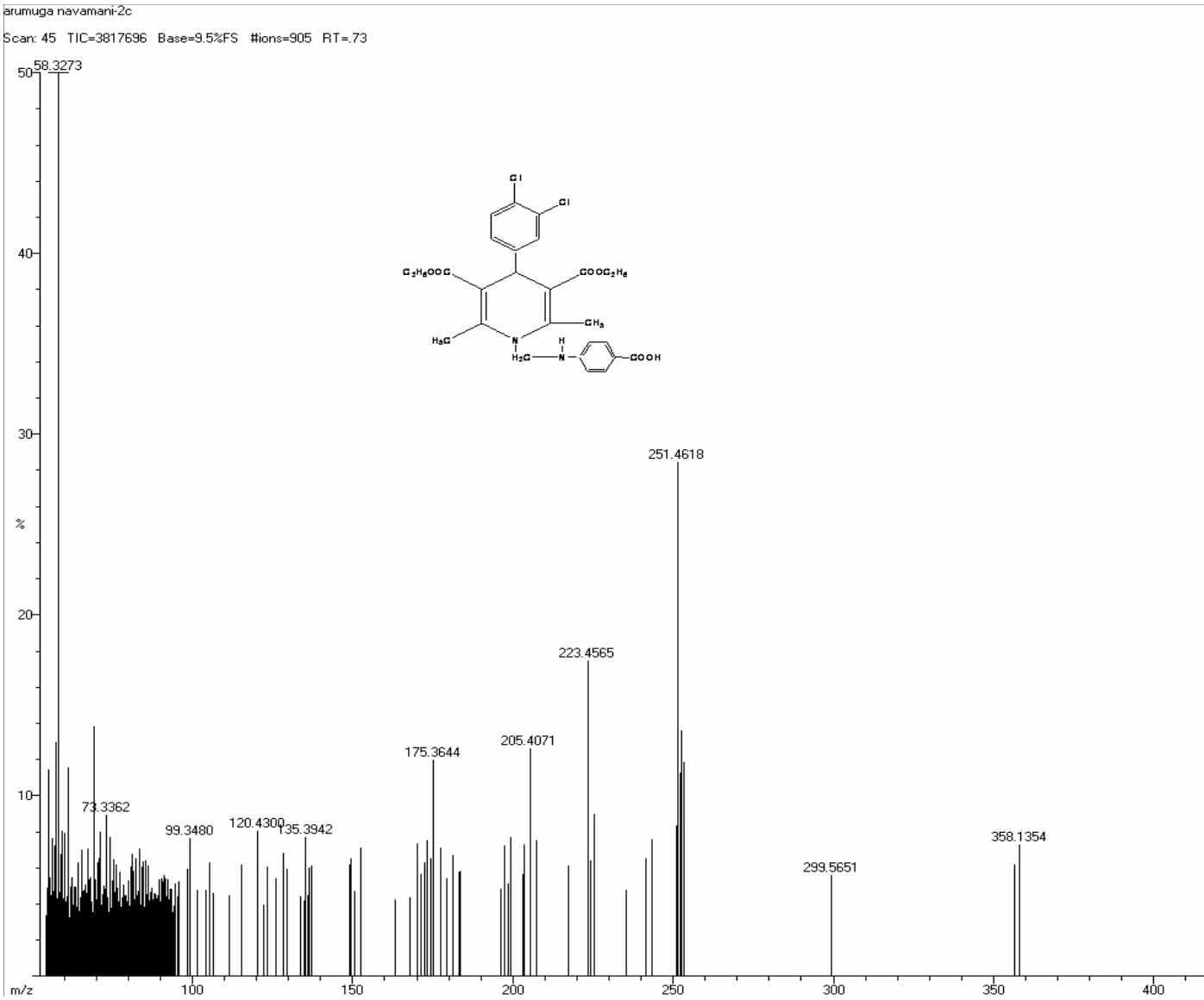


Fig-19 MASS Spectra of Compound- 2d

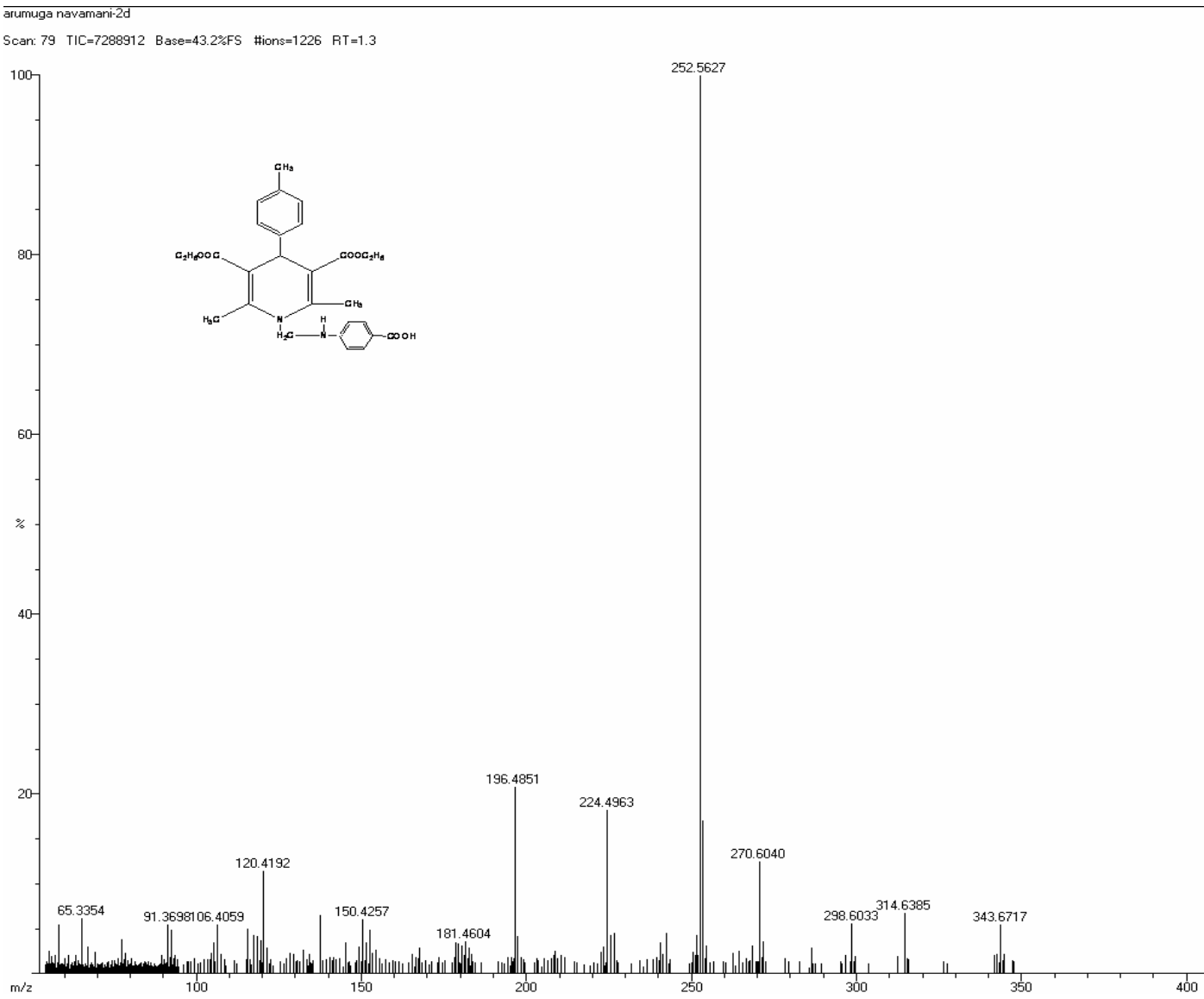


Fig-20 MASS Spectra of Compound- 2e

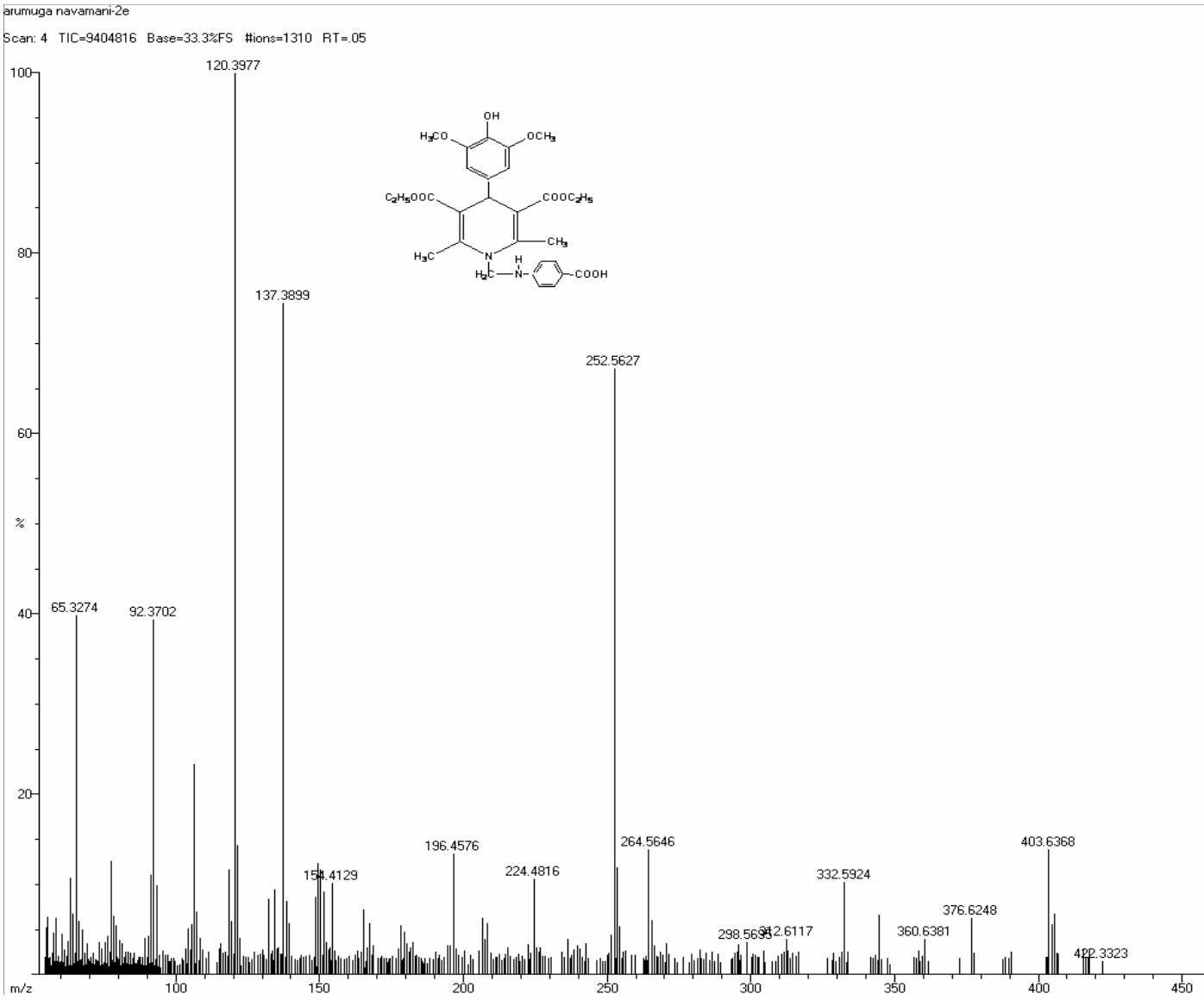

arumuga navamani-2e
Scan: 4 TIC=9404816 Base=33.3%FS #ions=1310 RT=.05
120.3977
137.3899
252.5627
65.3274
92.3702
154.4129
196.4576
224.4816
264.5646
298.5695
312.6117
332.5924
360.6381
376.6248
403.6368
422.3323
%
m/z
100
80
60
40
20
150
200
250
300
350
400
450

Table 6

^{1}HNMR Spectral data of the synthesized compounds

mpounds	Nature of proton	Aromatic proton (A r –H) (m)	N=CH-Ar(s)	-CH_3 (s)	C-O-CH_3(s)	Ar-OH	$N(CH_3)_2$ (s)	-OCH_3 (s)	-NH_2 (s)	To No pro
1a	No. of protonδ value (ppm)	5H 7.14-7.30	-	2H 1.71	-	-	-	-	-	
1b	No. of protonδ value (ppm)	5H 114.1-149.7	-	2H 16.3	-	-	-	2H 56.2	-	
1c	No. of protonδ value (ppm)	4H 4.43-7.16	-	2H 1.71	-	-	-	-	-	
1d	No. of protonδ value (ppm)	2H 4.43-6.94	-	3H 1.71-2.35	-	-	-	-	-	
1e	No. of protonδ value (ppm)	2H 5.96	-	2H 1.71	-	-	-	-	-	
2a	No. of protonδ value (ppm)	9H 6.87-7.30	-	2H 1.71	-	-	-	-	-	
2b	No. of protonδ value (ppm)	7H 6.46-8.05	-	2H 1.71	-	-	-	2H 3.73	-	
2c	No. of protonδ value (ppm)	7H 6.94-8.05	-	2H 1.71	-	-	-	-	-	
2d	No. of protonδ value (ppm)	8H 6.87-8.05	-	3H 1.7-2.78	-	-	-	-	-	
2e	No. of protonδ value (ppm)	6H 5.96-8.05	-	2H 1.71	-	1H 5.0	-	2H 3.73	-	

Fig.-21 ^{1}H NMR Spectra of compound 1a

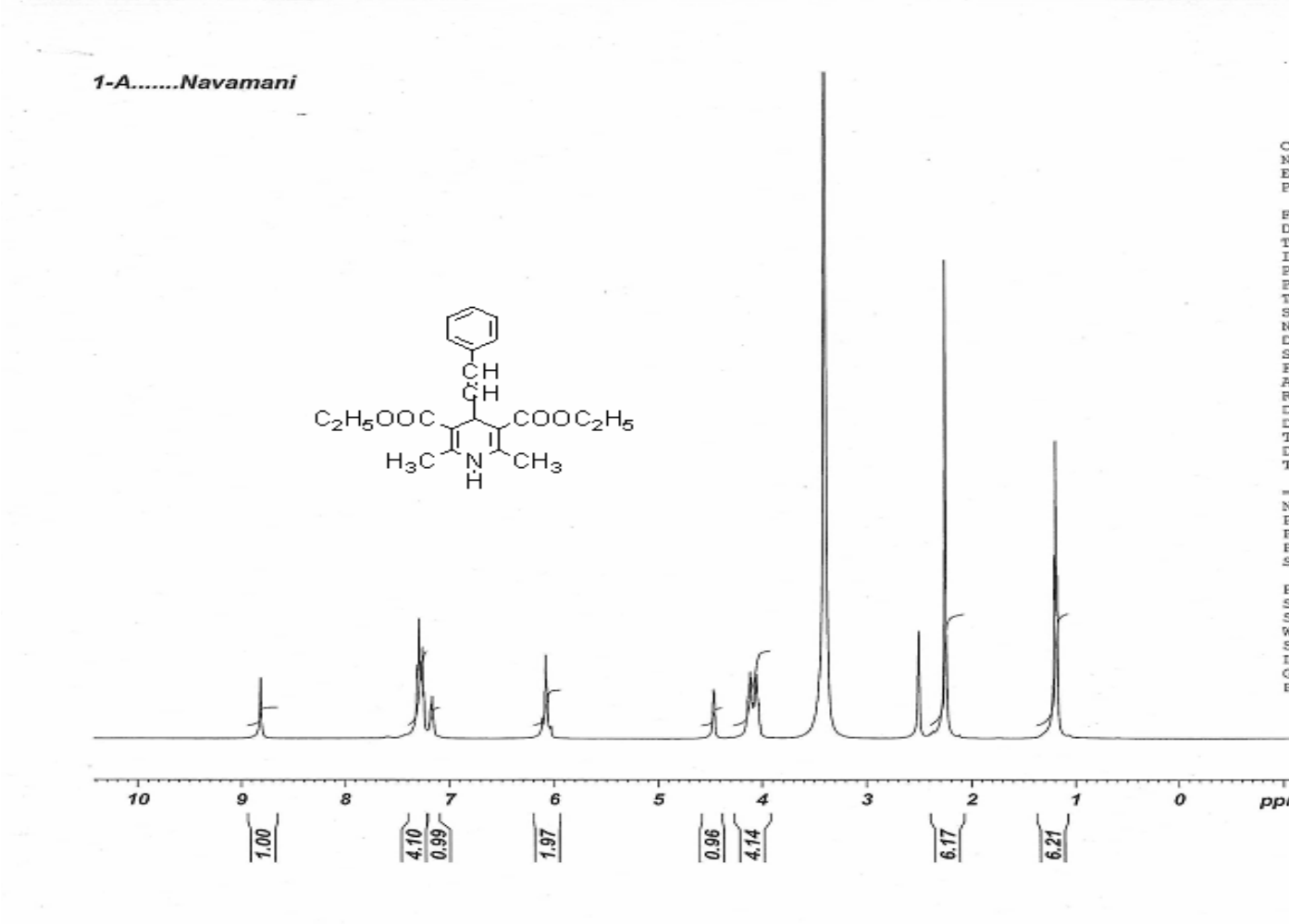


Fig.-22 ^{1}H NMR Spectra of compound 1b

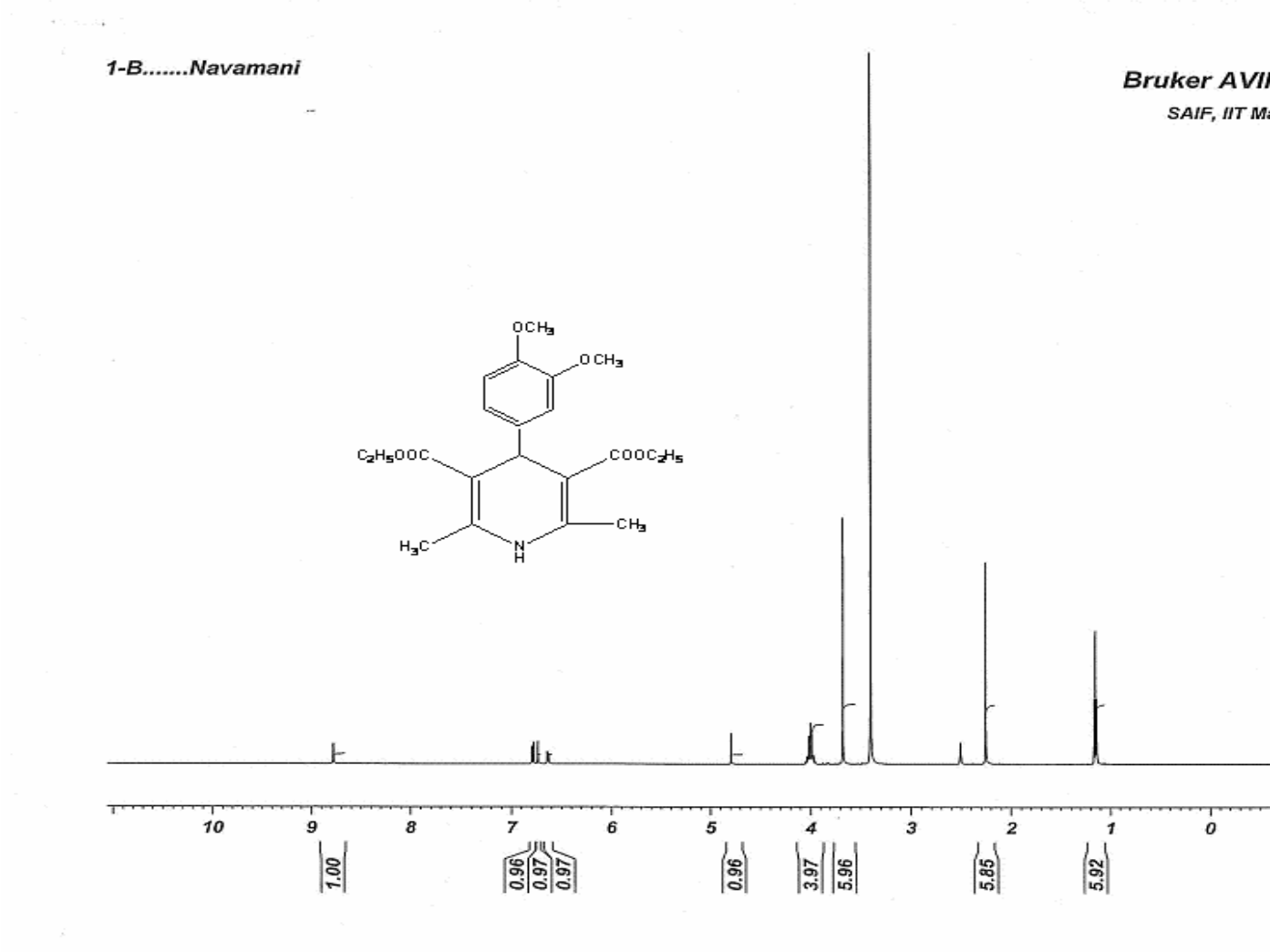


Fig.-23 ^{1}H NMR Spectra of compound 1c

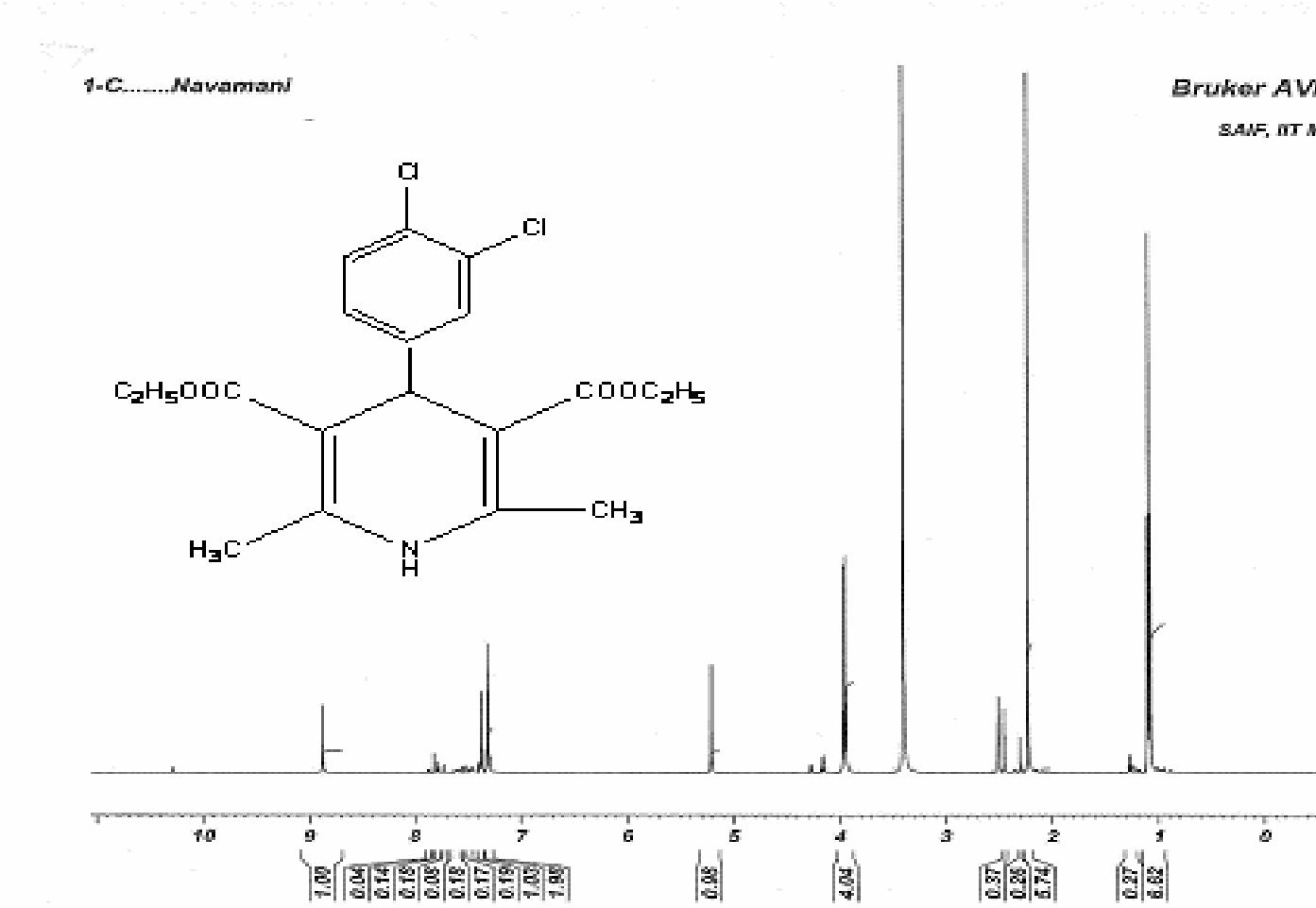


Fig.-24 ^{1}H NMR Spectra of compound 1d

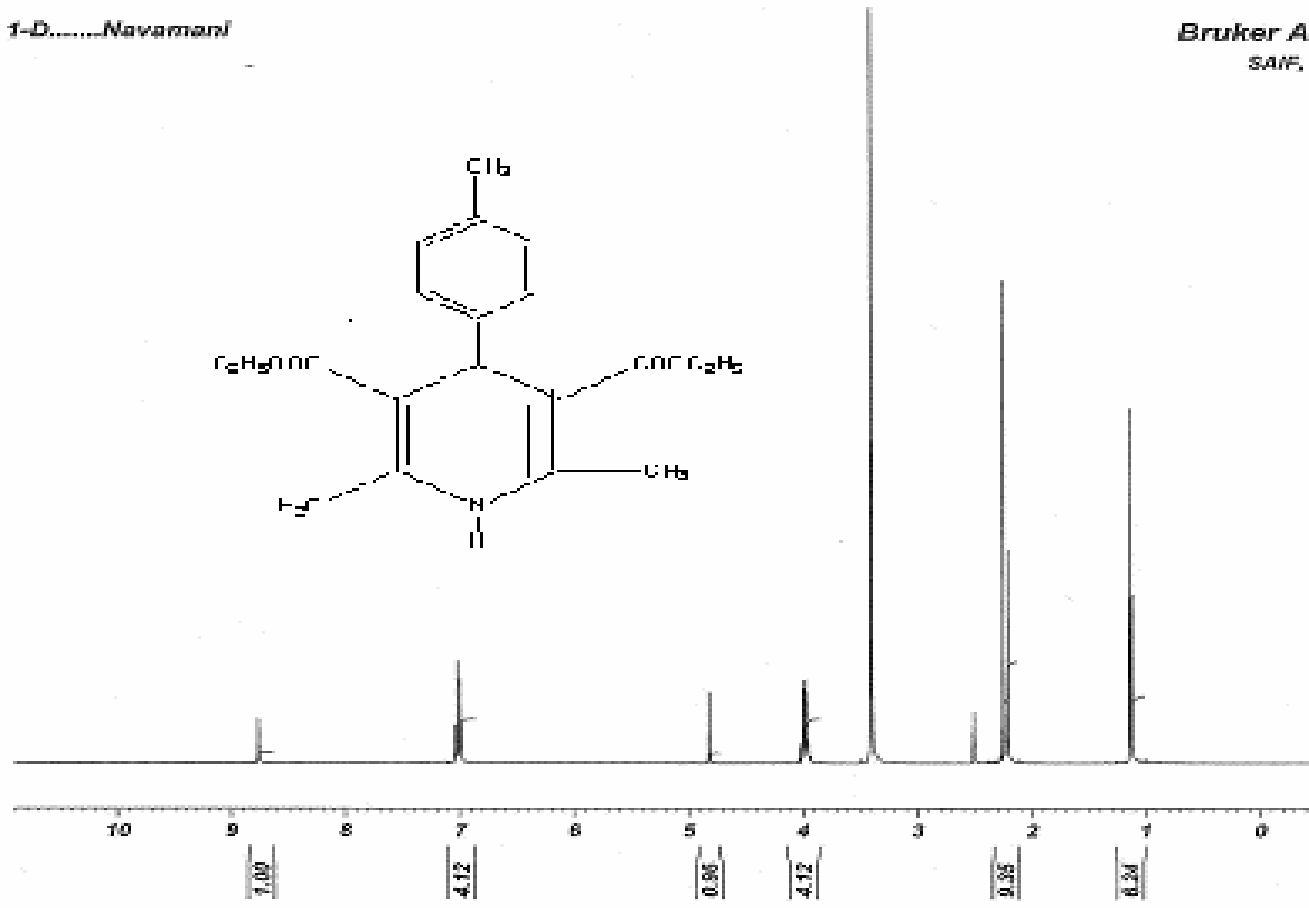


Fig.-25 ^{1}H NMR Spectra of compound 1e

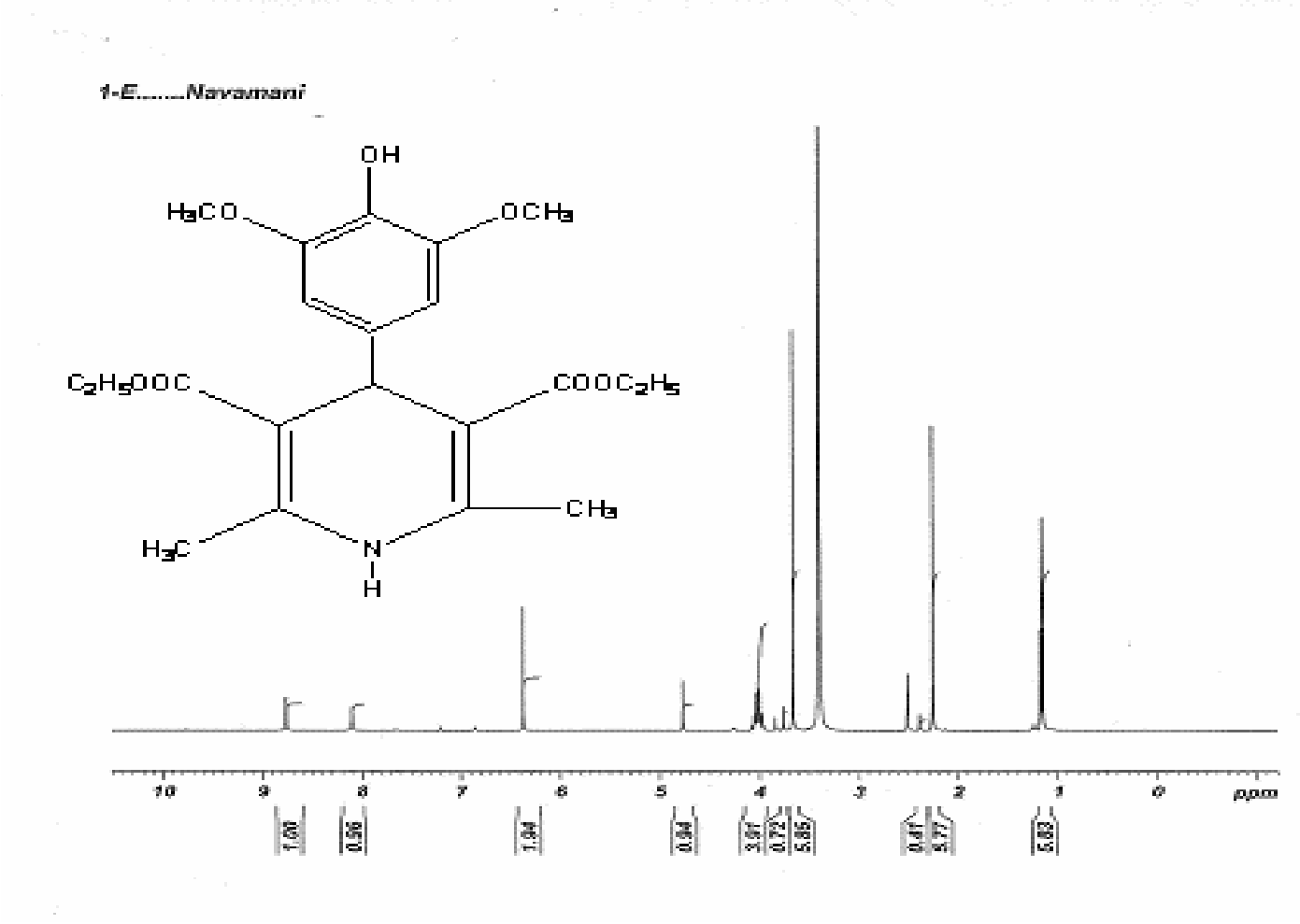


Fig.-26 ^{1}H NMR Spectra of compound 2a

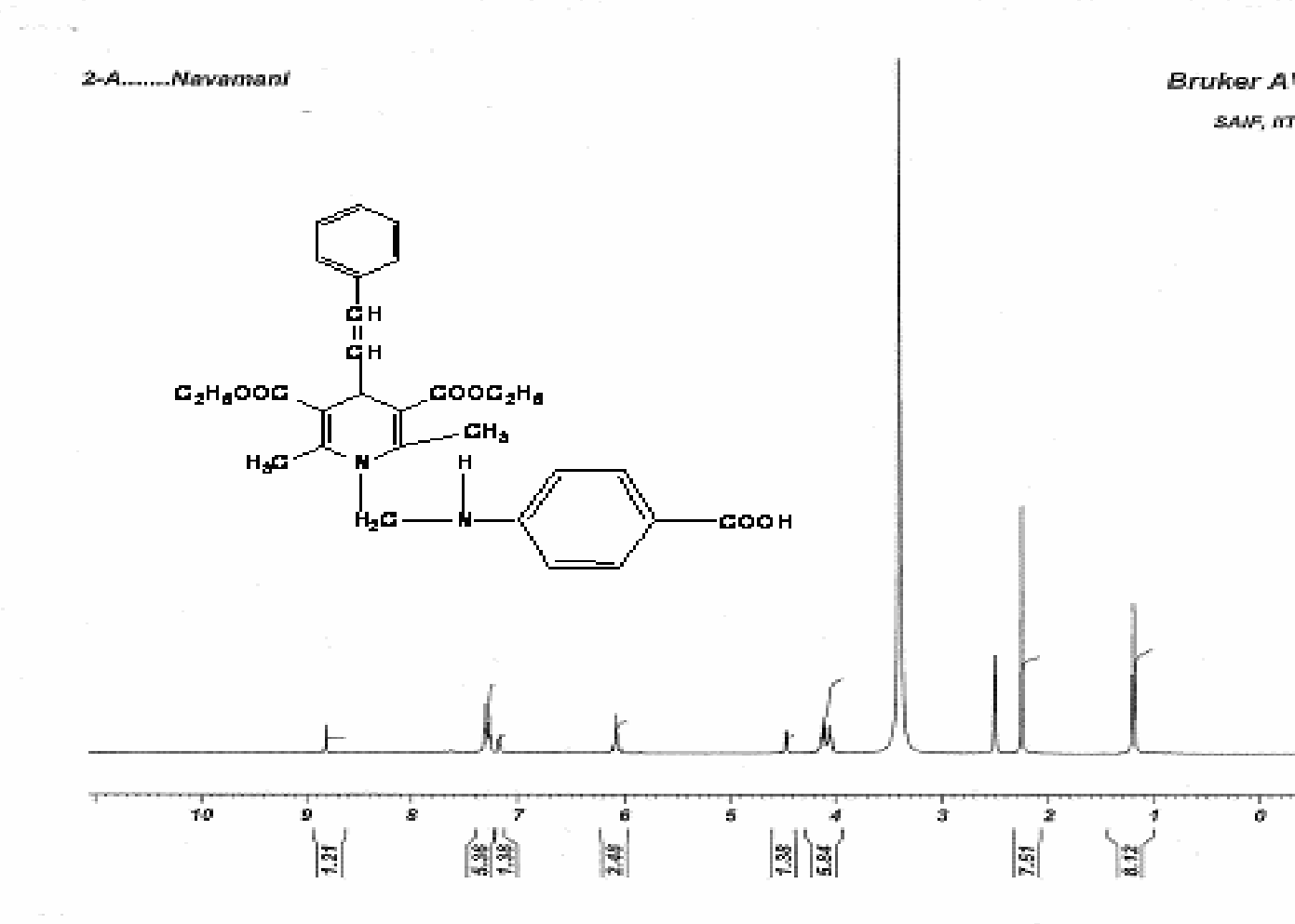


Fig.-27 ^{1}H NMR Spectra of compound 2b

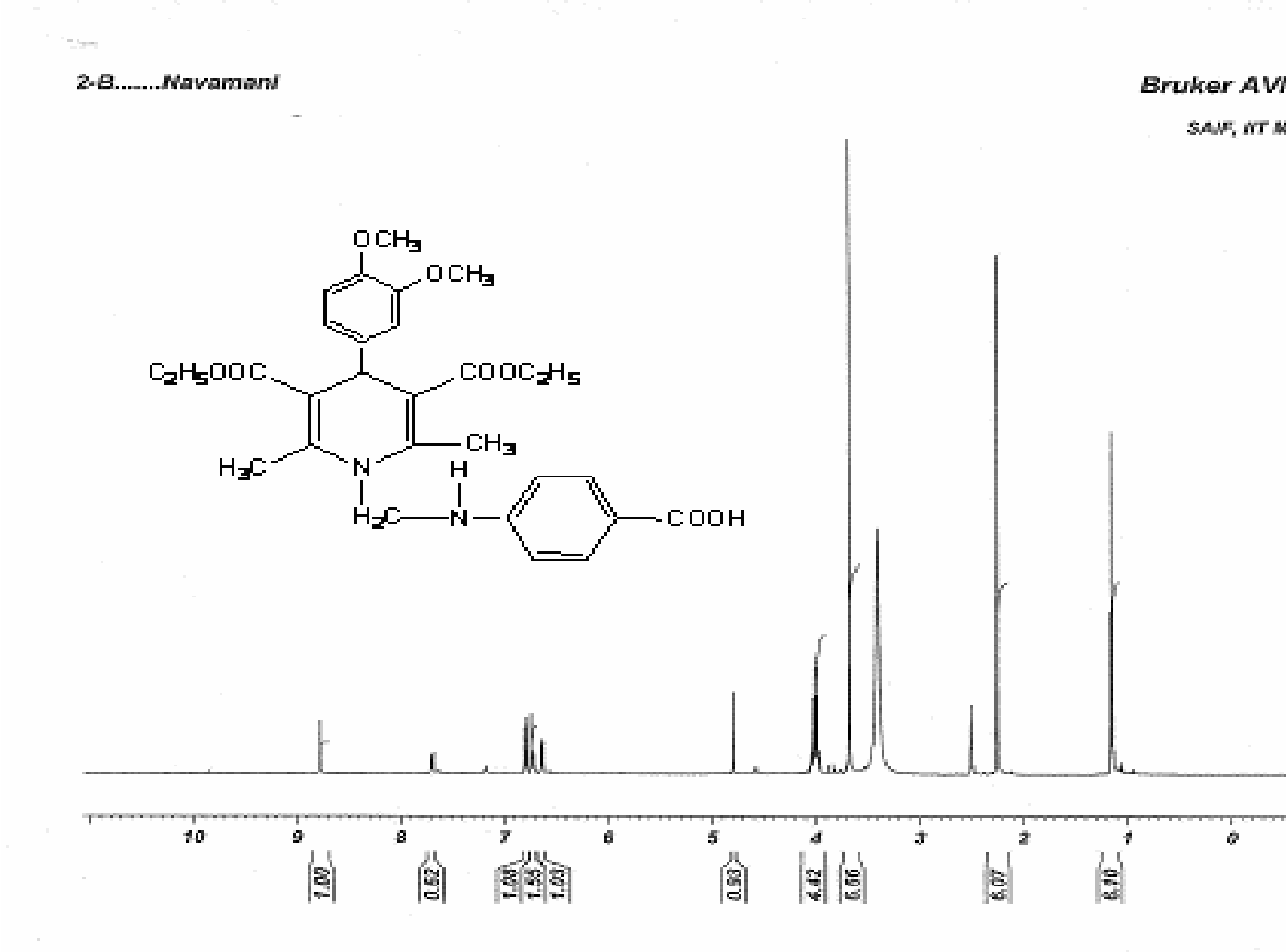


Fig.-28 ^{1}H NMR Spectra of compound 2c

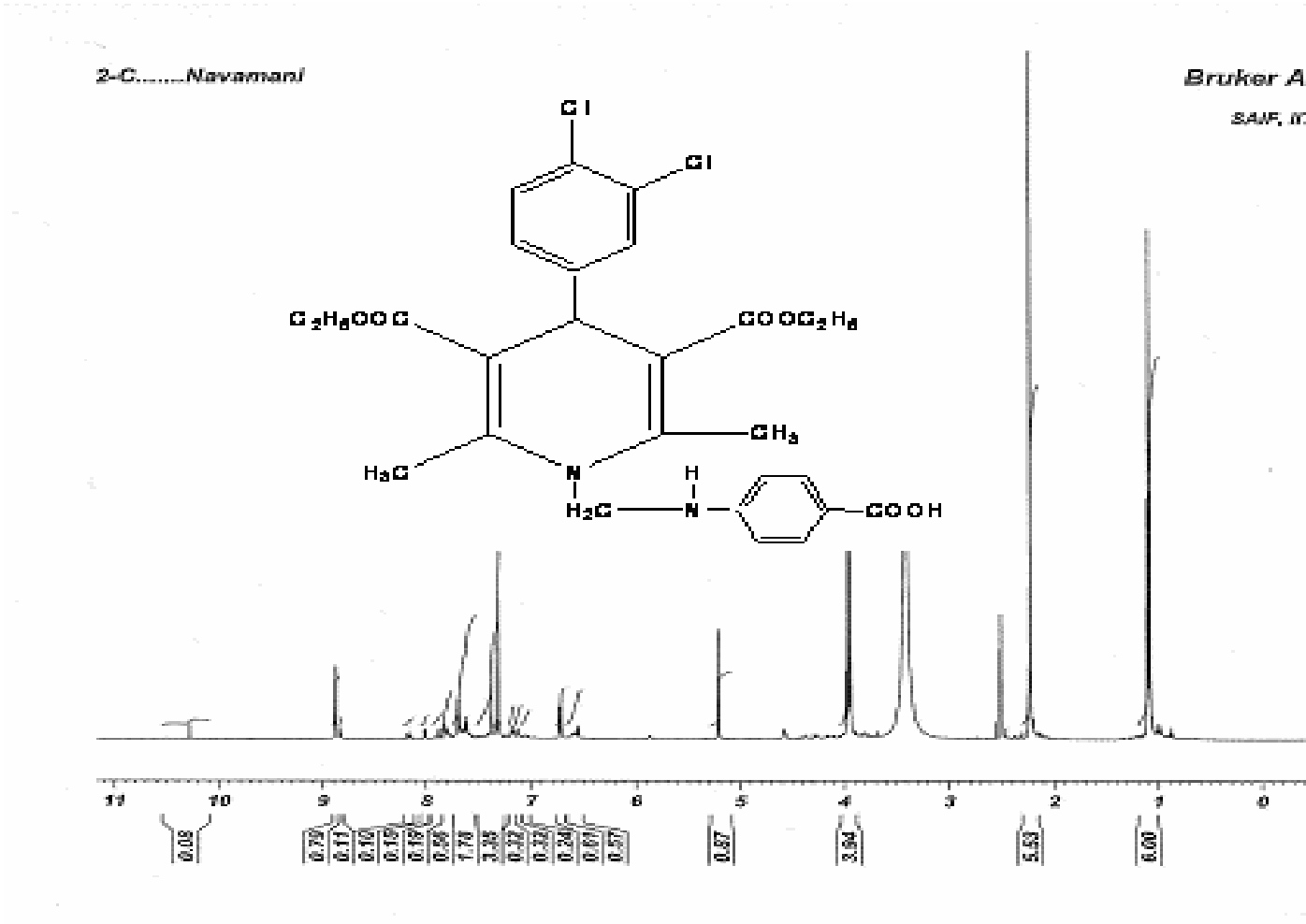


Fig.-29 ^{1}H NMR Spectra of compound 2d

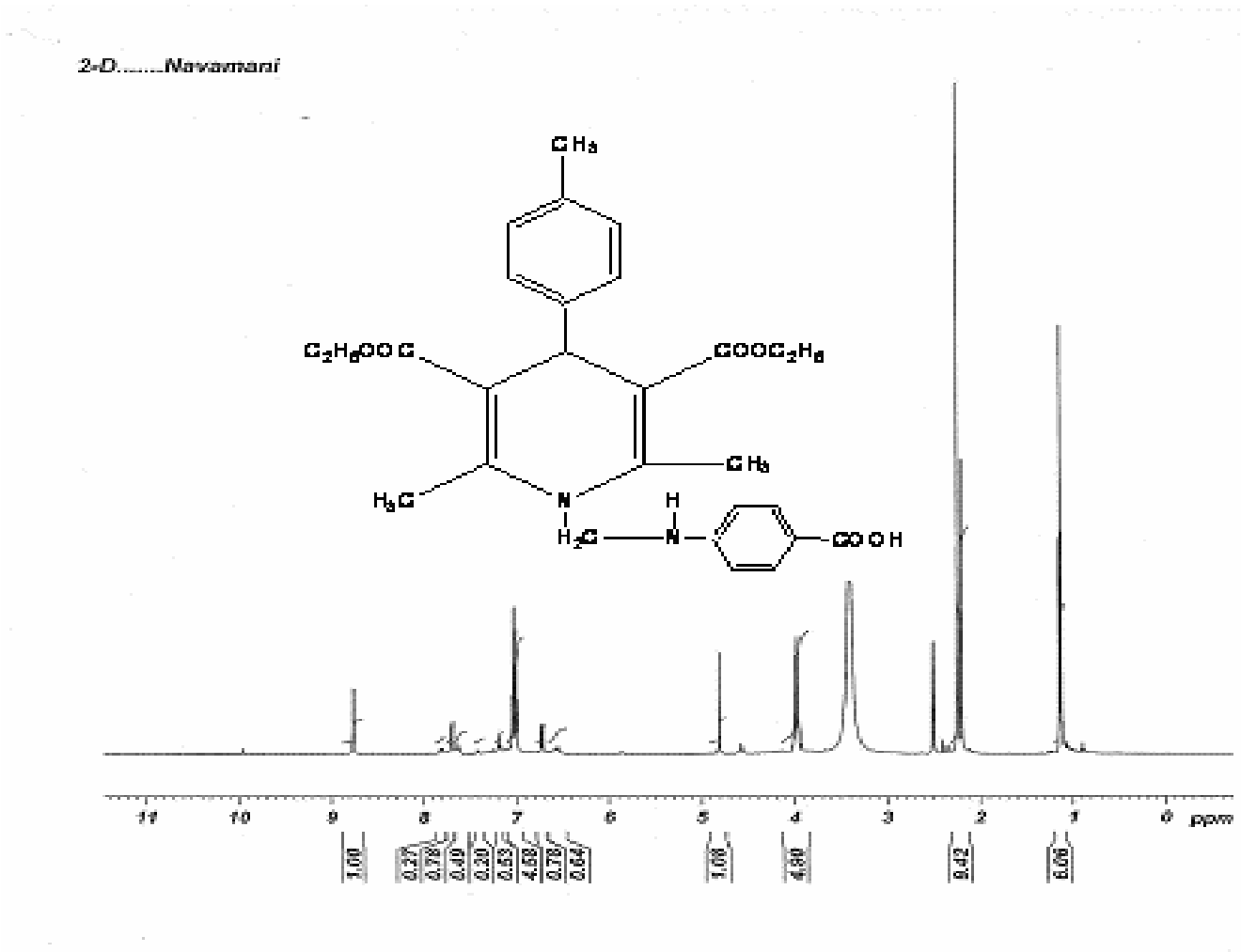
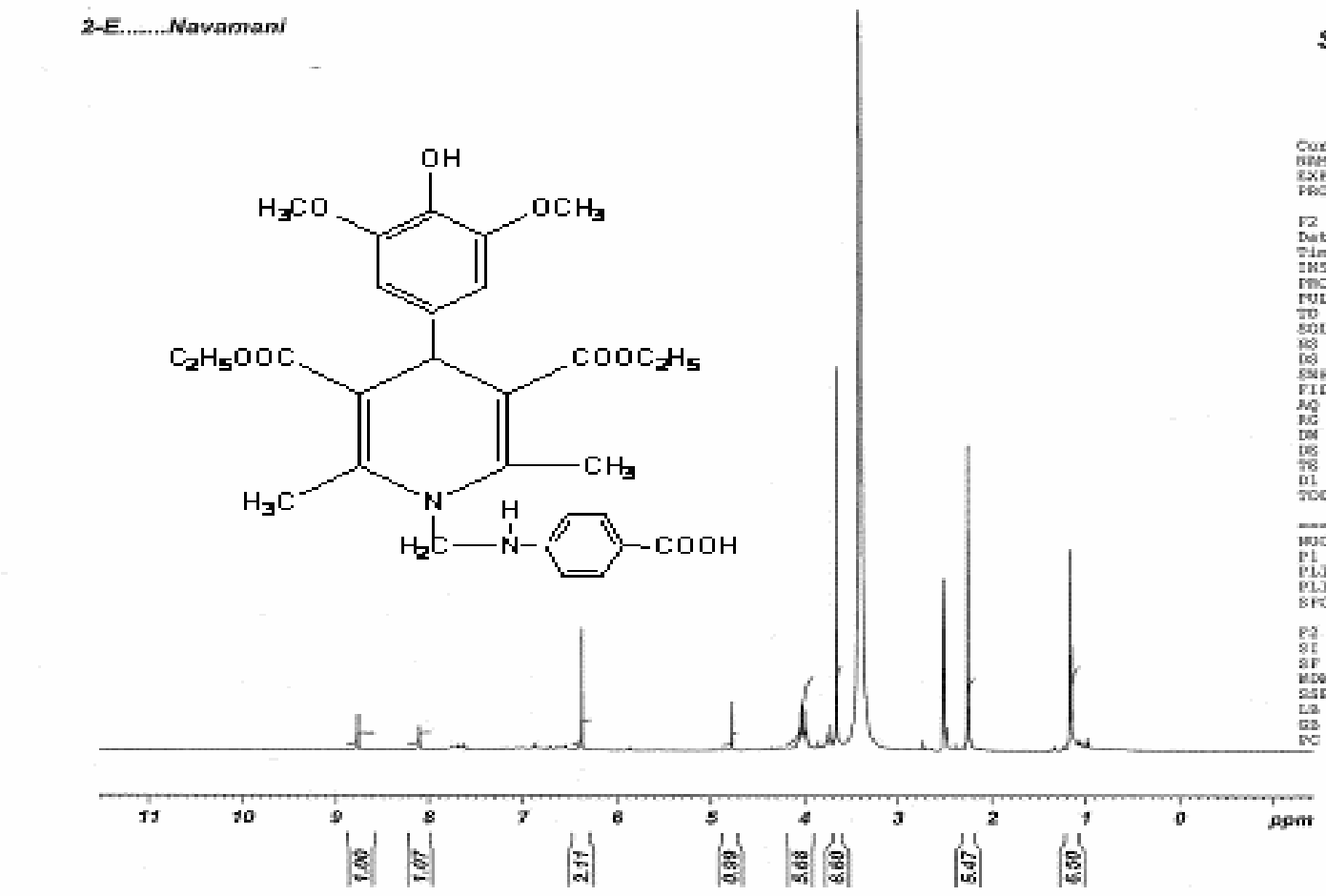


Fig.-30 ^{1}H NMR Spectra of compound 2e


2-E.....Navamani
OH
H3CO
OCH3
C2H5OOC
COOC2H5
CH3
H3C
N
H
H2C
N
COOH
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
1.07
2.11
0.99
5.68
8.60
5.47
6.89

PHARMACOLOGICAL EVALUATION

INVITRO ANTI-OXIDANT ACTIVITY

Antioxidants[36] are substances whose presence in relatively low concentration significantly inhibits the rate of oxidation of the major targets of oxidative activity viz., cell membranes and components, proteins and other cellular constituents.

Potential role of anti-oxidant in preventing the two important causes of premature death, cardiovascular diseases and cancer is partly attractive synthesis of some novel 1, 4-dihydro pyridine by using Biginelli condensation one pot-multi component reaction which are emerged as a novel anticancer agents.

ANTI-OXIDANT STUDIES

FREE RADICAL SCAVENGING ACTIVITY BY NITRIC OXIDE SCAVENING METHOD[37]

Chemicals used

- Sodium nitro prusside
- Phosphate buffer
- Methanol
- Griess reagent

Procedure

Nitric oxide scavenging activity was measured by spectrophotometry method. Sodium nitroprussicle (5mmol) in phosphate-buffered saline was mixed with a control test compound, but with an equivalent amount of methanol. Test solution at different concentrations (5- 100 mg/ml) were dissolved in methanol and incubated at 25^0c for 30mts. After 30min, 1.5ml of the incubated solution was removed and diluted with 1.5ml of griess reagent (1% sulphanilamide, 2% phosphoric acid, 0.1% naphthyl

ethylenediamine hydrochloride). The absorbance of the chromophore formed during the diazotization of the nitrite with sulphanilamide and the subsequent coupling value is the concentration of sample required to inhibit 50% of nitric oxide radical all determinations were performed in triplicates % inhibition of nitric oxide radical was calculated by following formula.

$$\text{Nitric oxide scavenged (\%)} = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

Where,

A_{cont} is the absorbance of the control reaction mixture.

A_{test} is the absorbance of the control sample of the synthesized compounds at different concentrations.

The antioxitdant activity of the synthesized compounds was expressed as IC_{50}Values.

Hydroxyl radical scavenging activity[38]

Chemicals used

- EDTA
- $Fecl_3$
- H_20_2
- Thio barbituric acid
- Tri chloro acetic acid
- Distilled water
- Ascorbic acid
- Deoxy ribose
- Phosphate buffer

Procedure

The scavenging capacity for hydroxyl radical was measured according to the modified method. The assay was performed by adding 0.1 ml EDTA, 0.01 ml $Fecl_3$, 0.1ml H_20_2 , 0.36ml of deoxy ribose. 1.0ml of test solutions (5 – 100 kg/ml) dissolved in distilled water,0.33ml of phosphate buffer (50mm, pH 7.4), and 0.1ml of ascorbic acid in sequence. The mixture was then incubated at 37^0 for 1 hour. A 1 ml portion of the incubated mixture was mixed with 1 ml of 10% TCA and 1 ml of 0.5% TBA to develop the chromogen which was measured at 532nm. BHT was used as a positive control.

% inhibition of hydroxyl radical was calculated by following formula.

$$\text{Hydroxyl radical scavenged (\%)} = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

Where,

A_{cont} is the absorbance of the control reaction mixture.

A test is the absorbance of sample of the synthesized compounds at different concentrations.

Table 7

***In Vitro* Nitric oxide scavenging activity of compounds**

S. No.	Compounds	%RSC				IC_{50}
		25µg/ml	50µg/ml	75µg/ml	100µg/ml	
1	**1a**	7.15±0.07	13.59±0.09	22.61±0.13	39.02±0.01	>100
2	**1b**	18.71±0.25	29.00±0.32	40.19±0.08	51.00±0.001	98.03
3	**1c**	14.86±0.23	28.57±0.09	39.48±0.12	50.30±0.19	99.22
4	**1d**	19.43±0.14	30.22±0.1	43.00±0.37	54.22±0.23	92.21
5	**1e**	26.39±0.12	52.79±0.2	72.28±0.19	82.00±0.09	47.35
6	**2a**	13.28±0.12	27.86±0.09	38.27±0.2	51.82±0.3	96.48
7	**2b**	16.90±0.08	31.00±0.019	44.08±0.13	66.12±0.09	75.62
8	**2c**	31.71±0.07	50.64±0.12	67.95±0.19	81.00±0.24	49.36
9	**2d**	34.00±0.09	59.81±0.21	73.39±0.25	84.56±0.16	41.79
10	**2e**	40.43±0.23	65.28±0.13	78.80±0.09	90.73±0.24	38.29

Table 8

***In Vitro* Hydroxyl scavenging activity of compounds**

S. No.	Compounds	%RSC				IC_{50}
		25µg/ml	50µg/ml	75µg/ml	100µg/ml	
1	**1a**	10.23±0.12	20.32±0.32	34.19±0.10	49.12±0.01	>100
2	**1b**	17.71±0.21	28.59±0.32	39.19±0.83	50.00±0.83	100
3	**1c**	10.49±0.02	19.96±0.4	33.83±0.09	51.96±0.1	51.66
4	**1d**	16.90±0.28	30.69±0.42	43.18±068	58.12±0.1	86.02
5	**1e**	23.28±0.56	67.86±0.35	79.27±0.81	92.82±0.3	36.84
6	**2a**	18.63±0.25	29.60±0.32	42.00±0.08	51.00±0.001	98.03
7	**2b**	19.43±0.14	31.22±0.1	48.36±0.34	62.22±0.28	80.36
8	**2c**	32.92±0.05	62.83±0.12	73.19±0.10	81.31±0.20	39.78
9	**2d**	38.23±0.03	65.19±0.17	75.28±0.3	84.31±0.9	38.34
10	**2e**	53.77±0.24	66.41±0.37	79.07±0.19	89.92±0.2	23.24

ANTIMICROBIAL ACTIVITY

Antibacterial Evaluation[39]

The antibacterial activity of different sample is done in disc diffusion method against the following organisms as directed by Ellen Jo Boron.

Escherichia coi	- Gram negative
Pseudomonas aeruginosa	- Gram negative
Bacillus cereuves	- Gram positive
Staphylococus auereus	- Gram positive
Media employed	- M.H.AGAR
Solvent control Used	- Dimethyl Sulfoxide

Test Samples

1a	Diethyl2,6-dimethyl-4-styryl-1,4-dihydro pyridine-3,5-dicarboxylate.
1b	Diethyl 4-(3, 4-dimethoxy phenyl) 2, 6-dimethyl-1, 4-dihydropyridine3,5-dicarboxylate.
1c	Diethyl4-(2,4-dichloro phenyl)- 2,6-dimethyl- 1, 4 –dihydropyridine-3,5-dicarboxylate.
1d	Diethyl 2, 6-dimethyl-4-p-tolyl-1,4-dihydropyridine-3,5-dicarboxylate
1e	Diethyl4-(4-hydroxy -3, 5-dimethoxy-2-phenyl)-2, 6-dimethyl-1,4dihydro pyridine-3, 5-dicarboxylate
2a	4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4-styryl pyridine-1(4h)-yl)methylamino)benzoic acid

2b 4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4- (3,4-dimethoxy phenyl)pyridine-1(4H)-yl) methylamino) benzoic acid.

2c 4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4- (3,4dichlorophenyl)pyridine-1(4H)- yl) methyamino) benzoic acid

2d 4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4-p-tolyl pyridine-1(4 H)-yl)methylamino) benzoic acid.

2e 4-(3,5-bis(ethoxy carbonyl)2, 6-dimethyl(4hydroxy3,5dimethoxypheny)pyridine1(4H)-yl)methylamino)benzoic acid.

Standard Used - Ciprofloxacin

The test samples used in concentration 1 mg / µl using dimethyl sulfoxide as solvent and 1mg/µl using their respective solvent Gentamicin, Ciprofloxacin and Tetracycline were used as standards for *Staphylococus auereus* and *Escherichia coli*.

Preparation of Media

Preparation of Nutrient agar

Peptone	- 0.5%
Sodium chloride	- 0.5%
Meat of beef extract	- 0.5%
Agar	- 3.0%
Distilled water	- q.s
pH adjusted to	- 7.2-7.4

Then the media is distributed in 5ml quantity into culture tubes and sterilized by autoclaving.

DISC DIFFUSION METHOD

To the serile nutrient agar suspension of E.coli was added to 45^{o}C and transferred to sterile petri dishes and allowed to solidify .sterile discs 5 mm in diameter (made for what man filter paper sterilized in isopropyl alcohol) dipped in solution containing compound samples and standard and blank paced on surface of agar plates.

Leave the plates standing for one hour at room temperature as a period of preincubation diffusion to minimize the effect of variation in time between the application different solutions. Then the plates were incubated at 37 ^{o}C for 18 hrs and observed for anti bacterial activity. The diameters of zones of inhibition were measured for plates in which zone of inhibition was observed and presented in **Table 9**.

The average area of zone of inhibition was calculated and compared with that of standard.

A similar procedure was carried out for studies of anti-bacterial activity of other sample against staphylococcus auereus, the results were tabulated in **Table 9**.

ANTIFUNGAL EVALUATION[39]

The antifungal activities of different samples were done in disc diffusion method against the following organism as described by Ellen Jo Boron.

1) *Aspergillus niger*

2) *Candida albicans*

Media employed - savouraud dextrose agar media

Solvent control used - dimethyl sulfoxide

The sample used in 1mg/µl concentration, using dimethyl sulfoxide as solvent and 1mg/µl concentration using their respective solvent Ketoconozole were used as standard against *Aspergillus niger*.

Test Samples

1a	Diethyl2,6-dimethyl-4-styryl-1,4-dihydro pyridine-3,5-dicarboxylate.
1b	Diethyl 4-(3, 4-dimethoxy phenyl) 2, 6-dimethyl-1, 4-dihydropyridine3,5-dicarboxylate.
1c	Diethyl4-(2,4-dichloro phenyl)- 2,6-dimethyl- 1, 4 –dihydropyridine-3,5-dicarboxylate.
1d	Diethyl 2, 6-dimethyl-4-p-tolyl-1,4-dihydropyridine-3,5-dicarboxylate
1e	Diethyl4-(4-hydroxy -3, 5-dimethoxy-2-phenyl)-2, 6-dimethyl-1,4dihydro pyridine-3, 5-dicarboxylate
2a	4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4-styryl pyridine-1(4h)-yl)methylamino)benzoic acid
2b	4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4- (3,4-dimethoxy phenyl)pyridine-1(4H)-yl) methylamino) benzoic acid.
2c	4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4- (3,4dichlorophenyl)pyridine-1(4H)- yl) methyamino) benzoic acid
2d	4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4-p-tolyl pyridine-1(4 H)-yl)methylamino) benzoic acid.
2e	4-(3,5-bis(ethoxy carbonyl)2, 6-dimethyl (4hydroxy3,5dimethoxypheny)pyridine1(4H)-yl)methylamino)benzoic acid.

Standard used Ketoconozole

Preparation of Media used

Savouraud Dextrose Agar medium	gm/l
Mycological procedure	10
Dextrose	40.0
Agar	15.0

Final pH at 25°C 5.6 ± 0.2

DISC DIFFUSION METHOD

Suspensions of *Aspergillus niger* were added to sterile nutrient agar at 45°C the mixture was transferred to sterile petri dishes and allowed to solidify .sterile discs 5 mm in diameter (made for what man filter paper sterilized in U.V lamp) dipped in solution containing compound samples and standard and blank paced on surface of agar plates.

Leave the plates standing for one hour at room temperature as a period of preincubation diffusion to minimize the effect of variation in time between the application different solutions.

The average area of zone of inhibition was calculated and compared with that of the standards and the results were tabulated 9.

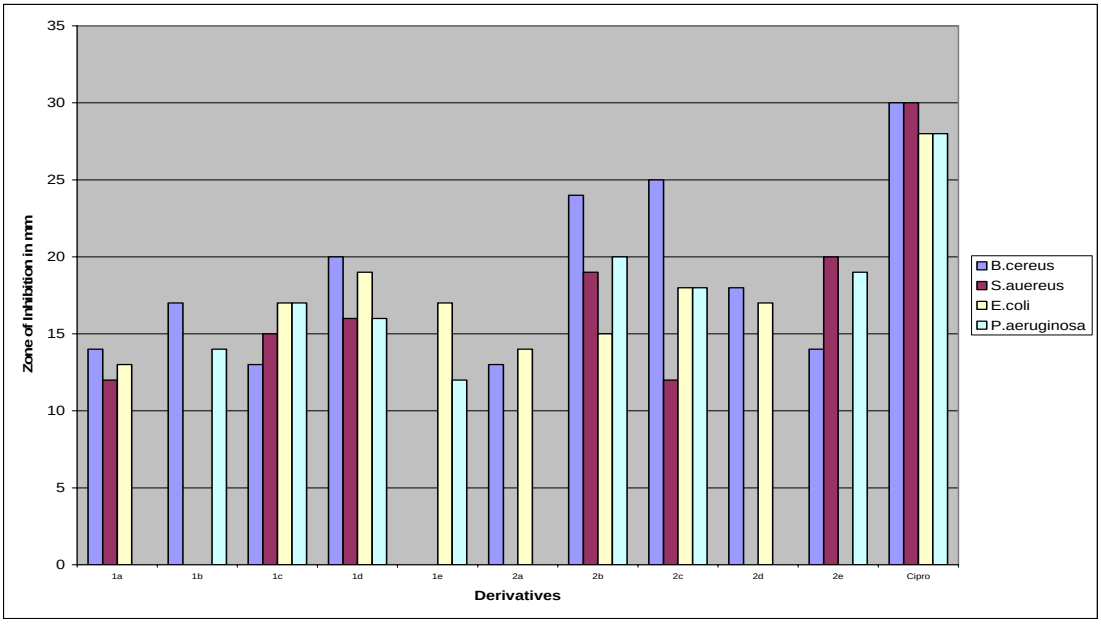
Table 9

Antimicrobial activity of synthesized 1,4-dihydropyridine derivatives

S. No.	Compound	Antibacterial activity zone of inhibition (MM)				Antifungal activity zone of inhibition (MM	
		B.cereus	***S.auereus***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
1	**1a**	14	12	13	-	12	18
2	**1b**	17	-	-	14	13	14
3	**1c**	13	15	17	17	15	19
4	**1d**	**20**	16	19	16	16	18
5	**1e**	-	-	17	12	12	17
6	**2a**	13	-	14	-	19	16
7	**2b**	**24**	19	15	20	17	14
8	**2c**	**25**	12	18	18	16	19
9	**2d**	18	-	17	-	14	15
10	**2e**	14	16	-	19	18	17

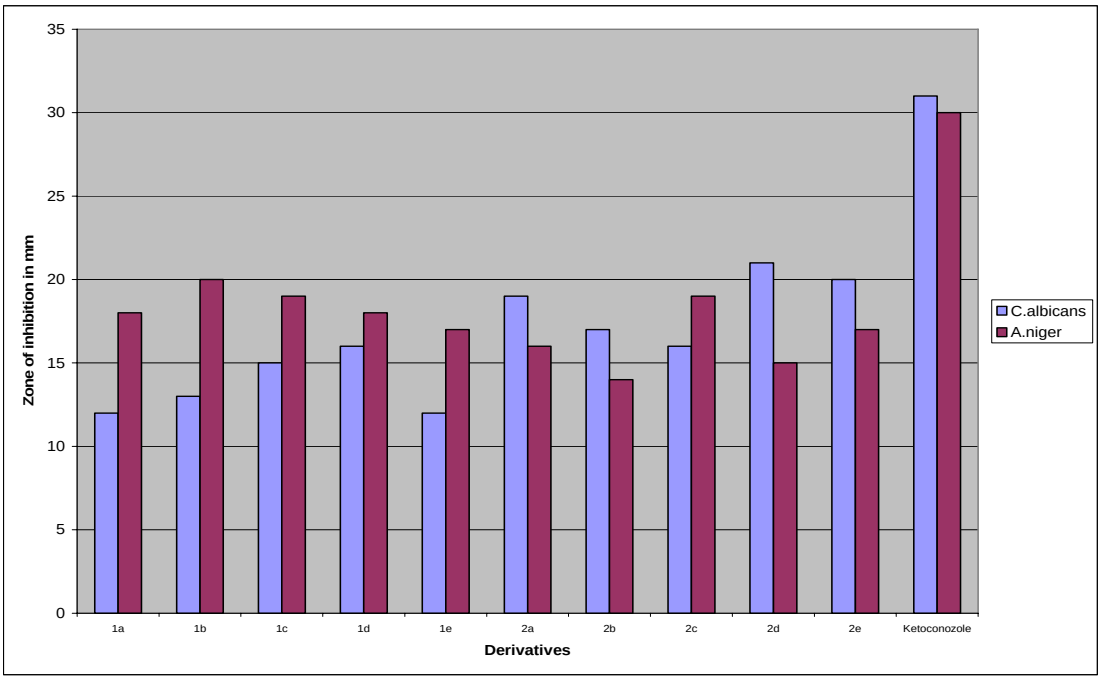
11	**Ciprofloxacin**	30	30	28	28	-	-
12	**Ketikonazale**	-	-	-	-	31	30

Fig. 31 Antibacterial activity of synthesised compounds 1a – 2e



BC = Bacillus Cereus ***EC = Escherichia Coli***
SA = Staphylococcus auereus ***PA = Pseudomonas Aeruginosa***

Fig. 32 Antifungal activity of synthesised compounds 1a – 2e



CA = Candida Albicans ***AN = Aspergillius Niger***

Photograph showing the effect of Cpd 1d against *S.auereus* and *A.niger*

Photograph showing the effect of Cpd 2d against *S.auereus* and *A.niger*

Photograph showing the effect of Cpd 2c against *S.auereus* and *A.niger*

RESULT S AND DISCUSSION

I have synthesized the 1, 4 dihydro pyridine derivatives with 65-70 % yield. It was further converted as substituted mannich base of 1, 4- dihydro pyridine derivative with an of 70-80% yield.

The melting point of all synthesized compounds was found in open capillary tubes and readings were uncorrected .the elemental analysis was determined and the results were tabulated in the **Table 3.** The found value of the element by elemental analysis closer to calculated values

The IR spectra of the compounds were done in a schimadzu FT 8300 infrared spectrophotometer (Vmaxcm-1) by using KBr discs. The results of IR spectra given in the **Table 4** shows that the functional groups such as phenolic hydroxyl, ,chlorine, amine, phenyl and methoxy groups may be present in the synthesized compound.

The ^{1}HNMR spectra of the synthesized 1, 4-dihydropyridine derivative were recorded on JEOL GSX 400 spectrometer using TMS as internal standard (chemical shifts in δ, ppm) and DMSO as the solvent The results of the ^{1}HNMR spectra given in **Table 6** shows that the numbers of hydrogen atoms present in all the synthesized compounds were exact when compared to the number of hydrogen atoms in the expected compounds.

The Mass spectra of the all synthesized compounds were done on a JEOL MSMATE spectrometer. The results were presented in **Table 5** show that molecular mass of the synthesized compounds were nearer to the molecular mass of expected compounds.

The synthesized compounds were screened for there *in vitro* antioxidant activity by Nitrous oxide, Hydroxyl radical scavenging activity. The results obtained were tabulated in **Table 7, 8** were given as mean IC_{50}. All the synthesized compounds showed good anti-oxidant activity, out of all the synthesized compounds 1e, 2c, 2d, 2e showed

significant anti-oxidant activity, in all the method except the compound 1a which showed less when compared to that of the standard butylated hydroxyl toluene (BHT).

The bleaching of Nitric oxide, hydroxyl ion absorption is representative of the capacity of the test compounds to scavenging free radical independently. The result of my investigation revealed that the test compound is electron donor and could react with free radicals to convert them to more stable product and terminate radical chain reaction.

The compound (1e, 2c, 2d, 2e) substituted with electron donating groups like methoxy and hydroxyl showed higher anti-oxidant activity compared to others.

The synthesized compounds were screened for their anti-microbial activity by Disc diffusion method. The results were tabulated in **Table 9**. The results showed that the compounds 1d, 2b 2c having very good activity when compare that of standard drug (ciprofloxacin). Because of the presence of methoxy group in 3&4th position (2b), similarly (2c) presence of chlorine in 2&4th position and presence of methyl group in p-position (1d).

SUMMARY AND CONCLUSION

Molecular modification of simple and complex chemical entities may lead to biological active compounds. Different types of approaches are made to derive such compounds which exhibit selective pharmacological activity. Pyridine and their related compounds exhibited potent antioxidant and antimicrobial activity.

This research work was oriented towards the finding of newly 1, 4-dihydro pyridine derivatives with antioxidant and anti microbial activities. The different substitution of some 1, 4- dihydro pyridine was synthesized by aromatic aldehydes and ethyl acetoacetate followed by condensation reactions. Ten compounds have been synthesized and all the compounds were tabulated.

Using different analytical techniques, elemental analysis, IR ^{1}HNMR and mass spectroscopes. The results of this analysis showed that the expected different substituted some dihydro pyridine derivative were prepared.

The newly synthesized some dihydro pyridine derivatives were evaluated for their antioxidant and antimicrobial activity. The synthesized compounds 1e,1c,2d,2e showed effective antioxidant activity, also all the compound expect compound 1d.2b,2c was found to exhibit good antimicrobial activity . This clearly indicates that new dihydro pyridine derivatives can be effectively synthesized by the method mentioned in this study and these compounds exhibited significant antimicrobial and anti oxidant activities.

FUTURE PLAN

In conclusion, the present study reveals that some 1,4-dihydropyridine derivatives could be used as template for the future development through modification or derivatization to design more potent therapeutic agents.

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